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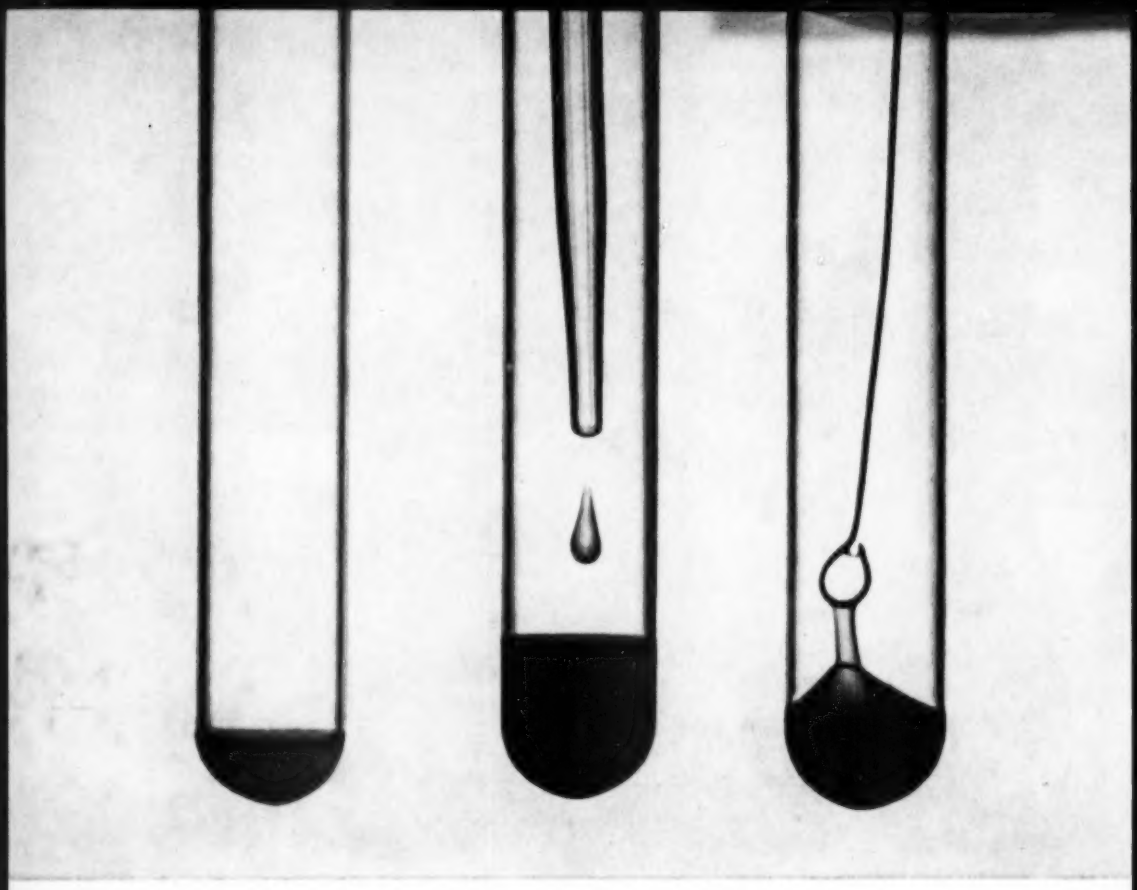

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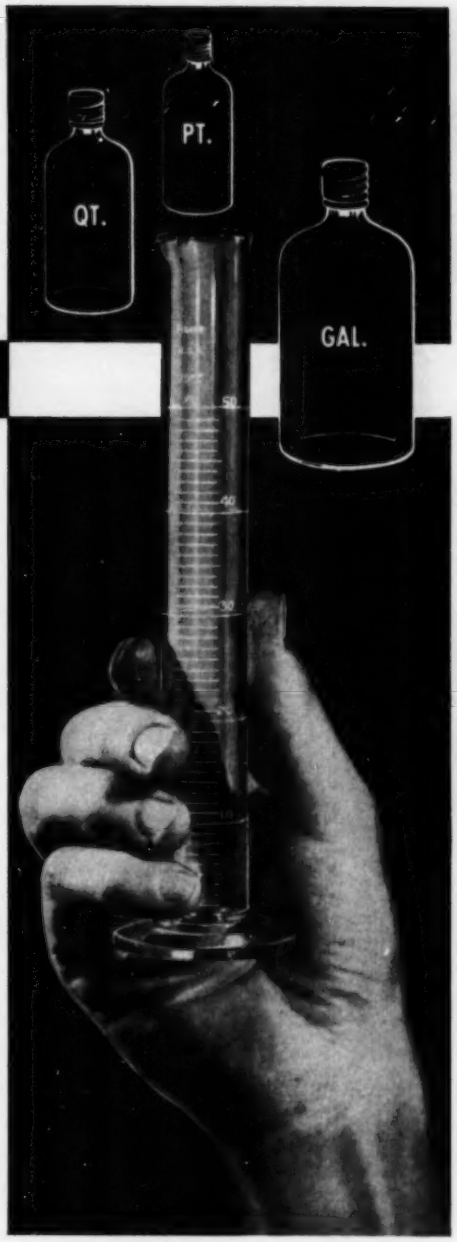

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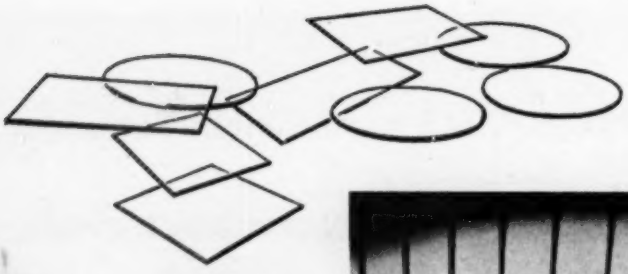

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FACTORS LEADING TO VARIATION IN CONCENTRATION OF "NEPHROTOXIC" ANTIGEN(S) OF GLOMERULAR BASEMENT MEMBRANE

CECIL A. KRAKOWER, M.D.
AND
SEYMOUR A. GREENSPON, M.S.
CHICAGO

THE STUDY of the antigenic composition of organs and tissues, with the exception of the constituents of blood, and free cells, such as spermatozoa, has been complicated by the admixture of various anatomical structures. Furthermore, such tissues and organs have not been too serviceable as test antigens in the employment of ordinary serologic methods, since they form unstable, and not very homogeneous, suspensions. Recourse to extracts has had worth while usage. However, the constituents of a tissue capable of being extracted by nondenaturing solvents form only a fraction, and at that a variable one, of the total tissue, particularly when one is dealing with poorly soluble intercellular matrices. Even the detection in vivo of antibodies to tissue components based on pathologic changes has had very limited applicability except where it pertains to tissue culture techniques and transplants.

It is, therefore, not surprising to find that most immunologic studies of adult tissues, as well as of their ontogenetic precursors, have been concerned chiefly with qualitative antigenic similarities and differences. Little is known, however, to what extent tissue, or even, for that matter, cellular, antigens vary quantitatively under different conditions of normal and abnormal function.

The use of the "nephrotoxic" antigen(s) has been most opportune in attempting to elucidate some of these problems. This antigen(s), in its most readily accessible form, is an integral component of the basement membrane of the glomerular capillaries of the kidney.[1] This membrane can be isolated as an anatomical unit virtually free of cellular contaminants. It has been shown to be related chemically to the collagens.* It is a membrane of extreme physiologic importance in renal filtration. Metabolically it probably has little activity,[4] in a manner comparable to collagens generally.[5] Nevertheless, it can undergo sharp changes under morbid states, reflected in abnormal filtration as well as in morphology. This membrane, in all likelihood, contains few antigenic groups, for, aside from the "nephrotoxic" antigen(s), it apparently has antigens in common with renal tubular basement membrane and connective tissue collagens.† Furthermore, the heterologous serum developed against the "nephrotoxic" antigen(s) will consistently produce a diffuse glomerulonephritis in the donor species. We have found repeatedly that a minimum

From the Department of Pathology, University of Illinois College of Medicine.

This investigation was aided by Grant H-1623 from the National Institutes of Health.

* References 2 and 3.

† References 6 through 8.

of approximately 5 mg. of normal adult canine glomerular basement membrane, or the equivalent of 0.02 mg. of basement membrane nitrogen, is necessary for the production of such an active serum in the rabbit. This constancy in the concentration of "nephrotoxic" antigen(s) in normal adult glomerular basement membrane made it possible to determine whether the concentration might vary with experimentally induced pathologic states of the kidney and in the course of the development of this organ.

PROCEDURE

Adult Kidneys.—These kidneys were obtained aseptically, either at the time the dogs were operated upon or at the end of a given postoperative period. This period was generally after 10 weeks. The kidneys were immersed in 0.85% saline in sterile glass containers. The containers were sealed and stored at —15 C. It has been found that refrigeration of the kidneys for a week or longer without prior perfusion is an effective means of preparation for the subsequent removal of blood and plasma components. After refrigeration the red blood cells become fragile and are eliminated by the repeated washings (with 0.85% saline) of the isolated glomeruli or their membranes derived by sonic vibration. These products, after cleaning, invariably give a negative benzidine test for hemoglobin. It is also easier to isolate glomeruli free of contaminants from nonperfused refrigerated kidneys than it is from ones that have been perfused.

Glomeruli were isolated from the kidneys in the manner described previously.[1] In brief, this consisted of removing the containers with the kidneys from the refrigerator at —15 C. the evening before they were to be processed and placing them in a refrigerator at 0 C. All operations were performed in a sterile room at ordinary room temperature. The containers were brought to room temperature only as needed, so that at the time of processing, the kidneys were cold, but not solidly frozen. The kidney capsules were stripped. The kidneys were halved sagittally. The medullae were removed with forceps and curved ophthalmic scissors. The cortex was "buttered" through a sterilized 150-mesh Monel metal screen‡ held taut in a special sterilized holder devised by one of us (S. A. G.). A flexible angled spatula was used for this purpose. The sievings were gently removed from the opposite surface of the screen with a separate sterile spatula and mixed with 0.85% saline in a sterile bowl placed under the holder. The suspension thus obtained was poured into a sterile test tube, which was corked and briskly shaken. Each kidney was processed separately, with use of a fresh screen for each and an individual numbered test tube. These tubes were centrifuged at 1,500 rpm for five minutes. The supernatant was discarded. The residue was shaken with isotonic saline and allowed to settle, while the tubes were kept in a chilled bath with ice. When settling of the glomeruli had occurred, the supernatant was withdrawn by suction. This process of resuspension, settling, and withdrawal by suction of the supernatant was repeated until by gross observation the suspension was made up of particles conforming in size and shape to glomeruli with no other contaminants. Samples from each tube were withdrawn with sterile precautions, placed on glass slides, covered with a glass slip, and examined microscopically. Only preparations with no or minimal contaminants were accepted. The usable preparations were combined and centrifuged. The firmly packed glomeruli were drained and weighed. They were then resuspended in a given volume of saline. The number of glomeruli and any contaminants, such as free parietal capsules, tiny sectors of tubules, or equally small bits of cortex, were counted in a hemacytometer on the same basis as a white blood cell count, using a solution of methylene blue as diluent. The values were determined per cubic millimeter. The percentage of glomeruli retaining their parietal capsules was determined on an ordinary mount on a glass slide. At least 200 glomeruli were inspected to obtain this percentage.

Neonatal Kidneys.—Kidneys were obtained from pups within the first 24 hours of life. They were removed aseptically. Their capsules were stripped. They were then immersed in sterile 0.85% saline in test tubes. The tubes were corked, sealed with paraffin, and placed in the deep

‡ F. P. Smith Wire and Iron Works, 2340 Clybourn Ave., Chicago.

freeze at —15 C. After freezing, all procedures were carried out as aseptically as possible in a cold room at 10 C., since these immature kidneys virtually liquefy once they have been brought to room temperature. The kidneys were kept frozen until the moment they were to be used. Hence, when removed from the deep freeze, they were stored temporarily either in a portable deep freeze unit or in an adjacent cold room at —10 to —15 C.

The bevel of a Bard-Parker blade is 0.5 mm. wide. With use of the full depth of the bevel of the blade, supported by an appropriate handle, a primary layer was shaved from the frozen kidneys. This 0.5 mm. layer represented the approximate thickness of the neoformative zone. A second layer, representing the midcortex, 0.5 mm. thick was then shaved. The kidney was then halved sagittally, and the juxtamedullary cortex was trimmed away from the medulla with curved ophthalmic scissors. These individual cortical shavings were placed in separate containers with sterile isotonic saline. They were repeatedly washed by centrifugation and decanting. They were then blended in a Waring Blendor for three to five minutes. The blended cortical material was again repeatedly washed and then packed by centrifugation, drained, and weighed.

In isolating glomeruli from the neonatal cortex, the neoformative layer was always removed. Glomeruli were isolated from the combined midzone and juxtamedullary layers or from the latter only. The procedure that was followed corresponded to that described for the adult kidneys with the following exceptions: A 200-mesh Monel metal sieve was used in place of the 150-mesh. In order to obtain accurate counts of the isolated glomeruli, the volume of isotonic saline added to the combined, packed, drained, and weighed product should not exceed 3 cc. Sterile silicone-coated pipettes (to reduce loss of glomeruli by adhesion to glass) were used in glomerular transfers and in aliquots withdrawn for inoculation of rabbits.

General Considerations.—In order to obtain glomerular basement membrane, the freshly isolated, cleaned glomeruli were sonically vibrated § for 20 minutes at a plate voltage of 130 and an output of 130 to 150 volts.‖ The vibrated glomeruli were centrifuged at 1,500 rpm for 5 to 10 minutes. The opalescent supernatant was drawn off sterilely and saved for inoculation. The gray, translucent sediment was washed several times with saline and then packed by centrifugation, drained, and weighed. The weighed membranes were brought to volume, and aliquots were used for inoculation of rabbits.

The aliquots of whole glomeruli, glomerular basement membrane, and vibrated supernatants were mixed with aluminum hydroxide jelly, prepared by the method of Tracy and Welker.[9] Equal parts of suspension and jelly were used. Albino rabbits, weighing 2,500 gm., were inoculated in each thigh with this suspension. They were bled from the heart at the end of three weeks. The serum was stored at —15 C. The serum from each rabbit was tested separately. Healthy medium-sized dogs of mixed breed, weighing 10 to 16 lb. (4.5 to 7.3 kg.), were inoculated intravenously with 1.5 cc. of serum per pound. The dogs were killed on the seventh day after inoculation. Urine was withdrawn, and a qualitative test for protein was performed, using Exton's reagent.[10] Complete autopsies were performed. In all instances sections of the kidneys were prepared for microscopic study. Careful microscopic studies of all organs were made in dogs with unilateral hydronephrosis and in pups which were inoculated with "nephrotoxic" serum.

Chemical Analyses.—Nitrogen determinations on fat-free dried material were performed according to the micro-Kjeldahl procedure of Ma and Zuazaga.[11] The ammonia was absorbed in boric acid according to Winkler's procedure.[12] Schneider's method, as modified in a later procedure, using hot perchloric acid in place of trichloroacetic acid, was used for the determination of DNA (deoxyribose nucleic acid).[13] The quantity of DNA was estimated by the spectrophotometric method of Dische,[14] using diphenylamine. Phosphorus was determined according to the procedure outlined by Umbreit, Burris, and Stauffer.[15] Blood N. P. N. and plasma proteins were determined by the methods of Folin and Wu [16] and an adaptation of Greenberg's method, respectively.[17]

§ Magnetostriction Oscillator, Model S-102, 50 watt, Raytheon Mfg. Co., Waltham, Mass.

‖ Whenever possible, the vibration should be performed on the same day as that on which the glomeruli are isolated.

Mathematical Considerations.—The amount of glomerular material lost to the supernatant after sonic vibration was determined by the difference in the original wet weight of the whole glomeruli and that of the wet weight of the sedimented basement membranes.

In order to estimate the amount of basement membrane derived from the tufts of adult glomeruli in the total sediment after sonic vibrations, the assumption was made that the non-nephrotoxic parietal capsules [1] lost virtually none of their substance to the supernatant. The parietal capsules are lined by a single thin layer of parietal epithelium in the intact glomerulus.

Fig. 1.—Upper, external and sectioned aspects of hydronephrotic kidney four weeks after ureteral ligation and one week after intravenous injection of "nephrotoxic" serum. Note absence of any gross features of glomerulonephritis.

Lower, contralateral kidney, by contrast, showing numerous petechial hemorrhages over the cortical surface, in the figure to the left, and in the swollen, hemorrhagically streaked cortex and corticomedullary junction of the sectioned aspect, to the right. These are the classic gross features of "nephrotoxic" serum glomerulonephritis.

These epithelial cells are readily lost when the parietal capsule is liberated. The thick parietal capsule on vibration yields largish, thick plates with little fragmentation into particles of such size as to remain in the centrifuged supernatant. The total number of parietal capsules bound to glomeruli or free was therefore multiplied by 0.16 γ. The latter figure was obtained from many glomerular preparations of varying parietal capsular content derived from dogs of the

usual medium size and mixed breed used in the laboratory. The percentage which the total weight of parietal capsules represented in terms of the whole vibrated sediment was deducted from the values used for inoculation. The same fraction was applied in estimating the amount of nitrogen, since it has been found that the percentage of nitrogen of fat-free dried vibrated material seemingly was independent of the numbers of parietal capsules.

The Positivity of a Serum.—In order to standardize our findings, serums which yielded scattered, and often sparse, glomerular lesions associated with no, or little, proteinuria were excluded. Only serums which yielded a more diffuse glomerulonephritis of the classic proliferative, exudative, and hemorrhagic type with, as a rule, a 1+ or greater proteinuria were accepted as positive. In general, if 60% or more of an adequate number of serums tested for any given inoculum were positive, that level of inoculum was regarded as a positive titer.

OBSERVATIONS ON ADULT DOGS

Unilateral Ureteral Ligation.—The right ureter was exposed a short distance beyond the hilus through a transperitoneal approach. A 1 to 2 cm. sector of the

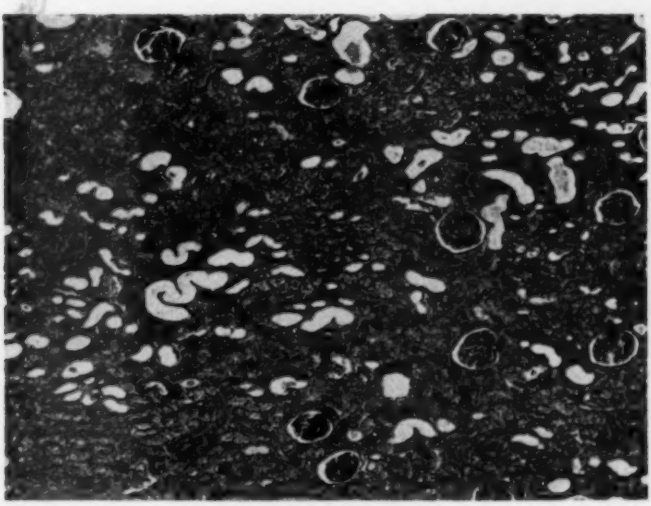

Fig. 2.—Microscopic appearance of hydronephrotic kidney, three weeks after ureteral ligation. Note the intact normal-appearing glomeruli, the dilated tubules, and the moderate degree of atrophy of the tubules elsewhere. Hematoxylin and eosin; × 68.

ureter was resected, and the proximal and distal ends were securely tied with cotton sutures. Groups of dogs so operated on were killed after either a 3- or a 10-week period. There were 24 dogs (20 female and 4 male) in the 3-week group, with an average weight of 13.5 lb. (9.5 to 16.5 lb.), or 6.15 kg. (4.3 to 7.5 kg.), and 33 dogs (26 female and 7 male) in the 10-week group, weighing 12.5 lb. (6.25 to 16.0 lb.), or 5.65 kg. (2.825 to 7.3 kg.). The moderately enlarged renal pelvis in the 3-week group contained 39.2 cc. of urine (15 to 150 cc.), whereas that of the 10-week group contained 115.6 cc. (39 to 260 cc.).

The three-week hydronephrotic kidneys were fairly tense, with prominent branching collateral veins over their capsular surfaces. The sectioned surfaces, as seen in Figure 1, revealed a moderately widened pelvis with some absorption of the single pyramid. There was a ridge along the convex border of the kidney with

approximately seven ridges running at right angles to it on each half of the kidney. These represented medullary trunks ordinarily entering the single pyramid. These ridges were pale, with occasional splashes of red. At times they contained brownish

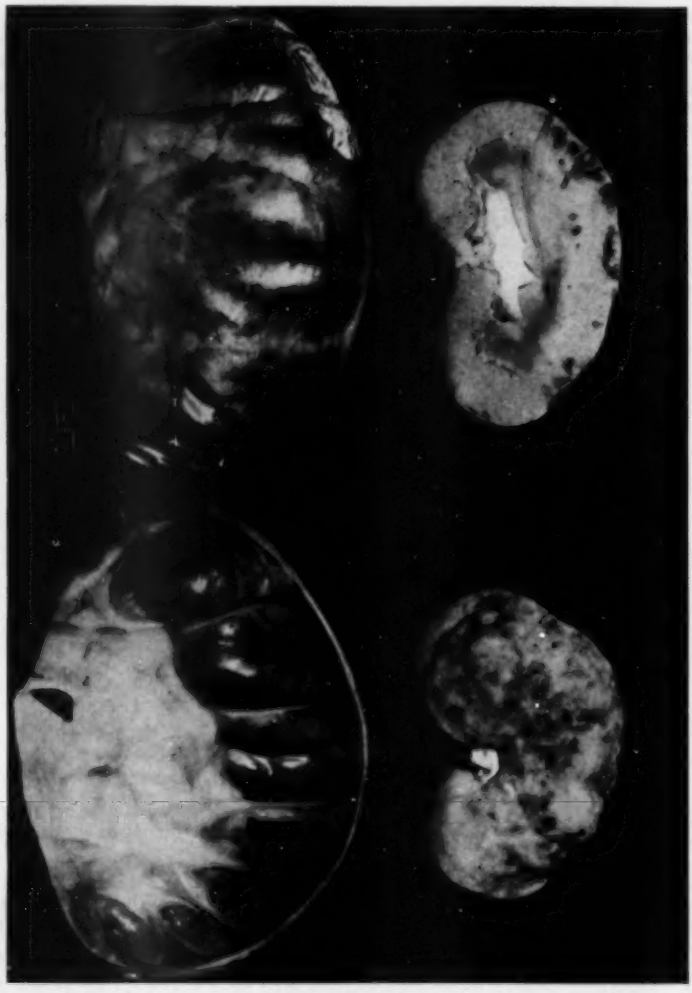

Fig. 3.—To the left is a large, hydronephrotic kidney 10 weeks after ureteral ligation, with its contralateral mate to the right. Seven days after intravenous injection of "nephrotoxic" serum, the hydronephrotic kidney fails to show any gross evidence of glomerulonephritis, while the contralateral kidney, externally and on section, presents blotchy areas of hemorrhage characteristic of experimental hemorrhagic glomerulonephritis.

deposits of hemosiderin. From the smooth, but thickened, pelvis near the hilus approximately six folds of mucous membrane emerged on each half of the kidney, tapering toward the convex ridge. These represented the usual pelvic extensions

about the medullary trunks as they enter the pyramid. There was deep hollowing of the columns of Bertini between the pelvic folds, and these columns were deep red. There was some reduction of the cortex, but with retained linear markings. The medulla was pale externally but pink to red toward the pelvis. Microscopically, these kidneys showed appreciable cortical atrophy in the hollowed and juxtahilar, anterior and posterior areas. There was better-preserved cortex overlying the medullary trunks and convex ridge, with dilated tubules often containing hyaline casts. The glomeruli were of almost normal appearance. There was little evident interstitial inflammatory cellular infiltrate (Fig. 2).

The 10-week hydronephrotic kidneys were, as a rule, very large and tense (Fig. 3). Capsular collateral veins were now considerably reduced in number and size. The external surface occasionally showed haustrations. The hugely enlarged pelvis presented a thin or thickish plateau over the convex area. The medullary trunks

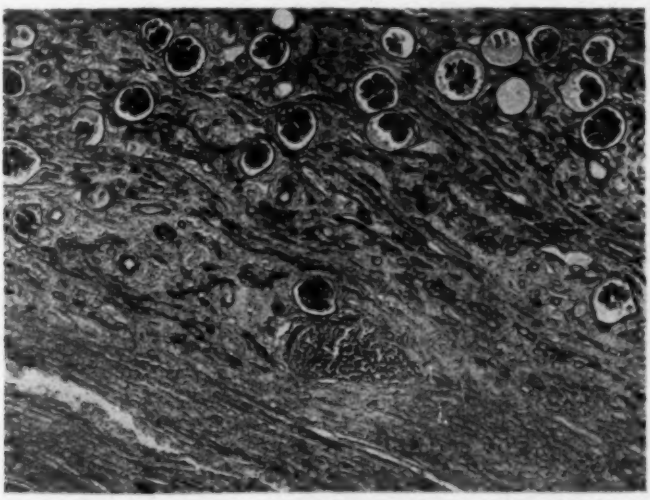

Fig. 4.—Microscopic appearance of hydronephrotic kidney 10 weeks after ureteral ligation. Note how the tubules are drawn outward, as are the glomeruli, to some extent. The latter show atrophic changes with some hydroglomeruli. Periodic acid-Schiff stain; × 68.

were in large part flattened and absorbed. The pelvic folds were tented, often folded, and sometimes bifid or trifid toward the convex extremity. The mucous membranes were thick and opaque. The cortex on section was greatly reduced in thickness and fawn in color, with loss of markings. The thinned medulla was deep red or livid. Microscopically (Fig. 4), the thinned stretched cortex presented markedly atrophic tubules with wrinkled, folded tubular basement membranes, not all of which were PAS (periodic acid-Schiff stain)-positive. There were PAS-positive casts in atrophic tubules of the corticomedullary junction and in the medulla. The basement membranes of the tubules in the compressed medulla stained poorly or not at all with PAS. The atrophic glomeruli had thickened, wrinkled basement membranes, but the parietal capsules were not appreciably thickened. There were occasional hydroglomeruli of varying degrees, at times with very atrophic glomerular tufts pushed to one side within a greatly distended capsular space. The atrophic glomeruli appeared more cellular but still contained red blood cells in some or all of their

capillary lumens. Occasional better-preserved glomeruli were seen, at times even associated with fairly well-preserved convoluted tubules. As in the three-week hydronephrotic kidney, there was usually an absence of interstitial inflammatory cellular reaction quite in contrast with the frequency of such inflammatory processes

TABLE 1.—*Principal Qualities of Kidneys and Glomeruli Derived from Them Three Weeks and Ten Weeks After Unilateral Ureteral Ligation*

	Three Weeks After Right Ureteral Ligation		Ten Weeks After Right Ureteral Ligation	
	Right Hydronephrotic Kidney	Left Kidney	Right Hydronephrotic Kidney	Left Kidney
Av. wt. of kidney, gm.	20.7 (16-34.5)	21.5 (15-29.1)	13.9 (8.5-23.6)	28.2 (19.8-53.8)
Av. thickness of renal cortex, cm.	0.4 (0.25-0.55)	0.7 (0.6-0.8)	0.2 (0.06-0.5)	0.6 (0.5-0.8)
No. of glomeruli isolated/kidney	291,067	360,389	174,230	473,950
Av. % of parietal capsules	22.5	31.7	42.5	14
% of tubular or cortical contaminants	Prep. 1:0	Prep. 1:0	Prep. 1:29.7 (used to titer whole glomeruli)	Prep. 1:0 (used to titer whole glomeruli)
	Prep. 2:0.8	Prep. 2:3.0	Prep. 2:0 (used to titer basement membrane)	Prep. 2:7.9 (used to titer basement membrane)
Av. wt. of glomerulus, γ	0.27	0.29	0.25	0.31
Av. size of glomerulus in stained mounts of isolated glomerular preparations, μ.	201.5 × 160.5	202.5 × 164.0	143.5 × 108.5	236.5 × 177.5
Av. volume of glomerulus, cu. μ obtained by hematocrit	20.2×10^4	19.5×10^4	12.5×10^4	19.3×10^4
Chemical composition, %, on fat-free dry wt. basis	—	—	0.98 phosphorus 11.7 nitrogen 4.95 DNA	1.2 phosphorus 12.3 nitrogen 4.6 DNA

TABLE 2.—*Titer of Antiserums to Whole Glomeruli Isolated from Kidneys Three Weeks and Ten Weeks Following Unilateral Ureteral Ligation*

	Three Weeks After Right Ureteral Ligation		Ten Weeks After Right Ureteral Ligation	
Amt. of Glomerular Material Inoculated into Rabbits, Mg.	Right Hydronephrotic Kidney No. Positive Serums (No. Serums Tested in Dogs)	Left Kidney No. Positive Serums (No. Serums Tested in Dogs)	Right Hydronephrotic Kidney No. Positive Serums (No. Serums Tested in Dogs)	Left Kidney No. Positive Serums (No. Serums Tested in Dogs)
5	—	—	2 (5)	1 (5)
10	0 (3)	0 (3)	0 (6)	2 (7)
15	2 (6)	5 (9)	6 (7)+*	4 (7)
20	0 (4)	3 (9)	—	5 (5)+
25	3 (7)	10 (11)+	4 (5)+	2 (6)
35	3 (5)+	—	5 (5)+	—
50	5 (5)+	3 (3)+	5 (5)+	—
60-120	7 (7)+	1 (2)	2 (3)+	—

* + = positive titers.

in the human hydronephrotic kidney. The marked thickening of the glomerular parietal capsule and arterial or arteriolar fibrosis common in the latter were largely absent in the dog.

Data relevant to these two sets of experiments at the different time intervals are given in Table 1.

It is apparent that in the first three weeks, despite the narrowing of the cortex in the hydronephrotic kidney, with associated parenchymal atrophy, there was relatively little difference in the aggregate size and weight of the isolated glomeruli and the size and weight of those of the opposite kidney. The latter presented little evidence of compensatory enlargement.

On the other hand, 10 weeks after ureteral ligation, the very much reduced cortex was now associated with marked reduction in size and volume of the isolated glomeruli and a more evident degree of hypertrophy of the opposite kidney, both in terms of the weight of the whole kidney and of the size of the glomeruli isolated from the kidney. There was little difference in the chemical composition of these two sets of glomeruli. The markedly reduced numbers of glomeruli isolated from the 10-week

TABLE 3.—*Titer of Antiserums to Twenty-Minute Sonically Vibrated Glomeruli Isolated from Kidneys Ten Weeks After Unilateral Ureteral Ligation*

	Hydronephrotic Right Kidney		Hypertrophied Left Kidney	
Amt. Membrane Material Inoculated into Rabbits, Mg.	Estimated Amt. Glomerular Basement Membrane (Estimated Nitrogen Content, Mg.)	No. Positive Serums (No. Serums Tested in Dogs)	Estimated Amt. Glomerular Basement Membrane (Estimated Nitrogen Content, Mg.)	No. Positive Serums (No. Serums Tested in Dogs)
2.5	1.1 (0.006)	4 (5)+*	2.0 (0.010)	3 (5)+
5.0	2.2 (0.011)	4 (5)+	4.0 (0.020)	1 (5)
10.0	4.4 (0.023)	3 (5)+	8.0 (0.040)	4 (5)+
Amt. Supernatant Material Representing Cellular Components Predominantly Inoculated into Rabbits, Mg.				
50	.....	2 (2)+	.....	0 (2)
125	.....	1 (1)+	.....	0 (2)

* + = positive titers.

hydronephrotic kidneys were an index, in part, of the loss of glomeruli in vivo, as well as, probably, loss in their preparation, since the thinned cortex was exceedingly tough and fibrous and was processed with difficulty.

At first, whole isolated glomeruli were titered for their "nephrotoxic" antigenic content. The results are presented in Table 2.

These results showed an interesting trend. In the three-week period, the glomeruli from the nonhydronephrotic kidneys, which at this stage showed little compensatory hypertrophy, yielded serums with a positive titer at the level of 25 mg. Previous studies have shown that normal glomeruli with a parietal capsular content greater than 19% will consistently produce serums with positive titer within the range of 25 mg. The glomeruli from the three-week hydronephrotic kidneys, however, despite a lower parietal capsular content, titered positively within the range of 35 mg., indicating a slight reduction in antigenic concentration.

This trend, however, was reversed in the 10-week period, in which the glomeruli from the hydronephrotic kidneys, despite an inordinately high content of contaminants and parietal capsules, yielded serums which titered positively at 15 mg., representing a considerable increase in antigenic concentration. The glomeruli of the opposite, nonhydronephrotic kidneys, now showing evidence of compensatory hypertrophy, presented an erratic end-point at 25 mg., although otherwise titering positively within the expected range of 15 to 20 mg. for glomeruli with a content of parietal capsules of less than 19%.

The results obtained for the 10-week period were borne out by titering the glomerular basement membranes (Table 3). This was done in order to eliminate possible interfering factors due to the cellular components of the whole glomeruli. There were apparent changes in the relative proportion of cells to basement membranes, as exemplified by the more cellular appearance of the atrophic glomeruli in the hydronephrotic kidneys, and there might conceivably have been some changes in the "non-nephrotoxic" antigenic make-up of these cells which might have interfered with the production of antibodies to the "nephrotoxic" antigen(s) of the basement membrane. It was for this reason that in all subsequent experiments of 10 weeks' duration, glomerular basement membrane was used for the purpose of determining the level of positive "nephrotoxic" antigenic titer.

The glomerular basement membrane derived from the 10-week hydronephrotic kidneys showed a definite increase in "nephrotoxic" antigenic concentration with strongly positive serums, at the level of an estimated 1.1 mg. of basement membrane and nitrogen content of 0.006 mg., by comparison with the normal of about 5 and 0.02 mg., respectively. The lowest limit of positive titer was not determined. The glomerular basement membrane from the opposite kidneys with compensatory hypertrophy showed erratic qualities, with a more definite level of positive titer at 8 mg. of basement membrane and 0.04 mg. of nitrogen, indicating a reduction in antigenic concentration.

The supernatants of centrifuged, sonically vibrated normal glomeruli, representing the cellular components of the glomeruli with the inclusion of some basement membrane material, generally yield positive serums within the range of 75 to 125 mg. or more. The positive values at 50 mg. for the glomeruli from the hydronephrotic kidneys were strongly indicative of increased fragility of the basement membranes of these atrophic glomeruli.

Herdman and Jaco [18] have recently shown by means of cineangiography that unilateral ureteral ligation in the rabbit leads to reduction in volume and rate of blood flow through the hydronephrotic kidney, increasing proportionally with the duration of the obstruction. Since the disturbed vascular flow is associated both with varied degrees of arterial ischemia and with obstruction to venous return, it was felt that these two elements should be investigated separately in attempting to interpret the above results.

Renal Ischemia Induced by Clamping the Right Renal Artery.—In 17 dogs the right renal artery was constricted by means of a Goldblatt clamp to the extent of an average 3.8 full turns of the screw of the clamp. At the end of 10 weeks, the clamped kidneys were grouped into three categories, viz., markedly atrophic, mildly atrophic, and nonatrophic. The markedly atropic kidneys came from eight dogs (five female and three male), weighing 17.2 lb. (13.0 to 21.5 lb.), or 7.8 kg. (5.9 to 9.75 kg.) at the time of operation and 17.6 lb. (12.5 to 22.0 lb), or 8.0 kg. (5.65 to 10.0 kg.) at the end of 10 weeks. Their preoperative blood pressures varied from 121 to 138 mm. Hg, with an average of 129 mm. Ten weeks postoperatively the average blood pressure was 153 mm. Hg (140 to 170 mm.).

The markedly atrophic kidneys were very small, presenting grossly reduced pelves and thinned cortices. The latter appeared either brownish or of yellow necrotic character. There were at times whitish, scarred-appearing zones. The corticomedullary junction often showed fibrosis. The small medulla was opaque white, occasionally with a pink cast. Microscopically, there were extensive areas

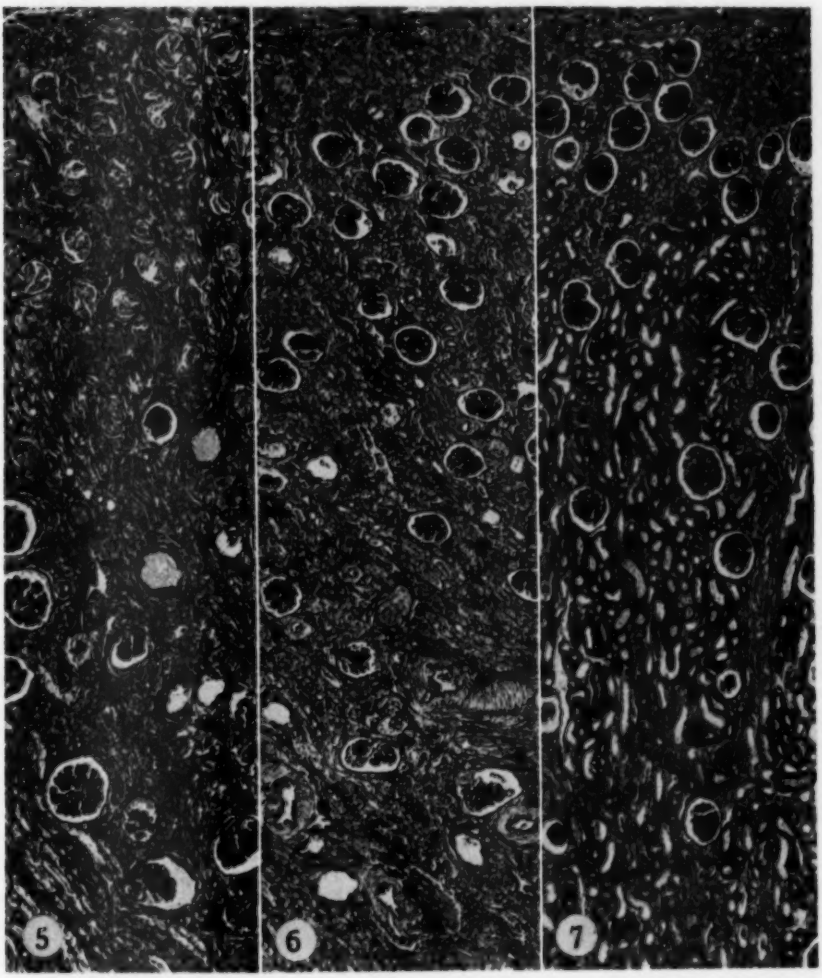

Fig. 5.—Microscopic appearance of markedly ischemic kidney 10 weeks after clamping the renal artery. Extensive subcapsular necrosis with acellular or parvicellular glomeruli but with well-defined collapsed basement membranes. Note the collapse and acellularity of the surrounding tubules. The juxtamedullary glomeruli are well preserved.

Fig. 6.—Section from kidney treated as that in Figure 5, but showing diffuse, marked atrophy of tubular structures with intact mildly atrophic glomeruli.

Fig. 7.—Microscopic appearance of mildly atrophic kidney 10 weeks after clamping the renal artery. Note subcapsular tubular and glomerular atrophy with well-preserved, more normal cortex elsewhere. All hematoxylin and eosin; × 68.

of necrosis occupying either the full thickness of the cortex or its subcapsular portions. These necrotic areas were characterized by (Fig. 5) collapsed acellular or parvicellular glomeruli, in which, however, the basement membranes of the tufts

TABLE 4.—*Principal Qualities of the Kidneys and Glomeruli Derived from Them Ten Weeks After Clamping Right Renal Artery*

	Markedly Atrophic Right Kidney	Corresponding Left Kidney	Mildly Atrophic Right Kidney	Corresponding Left Kidney	Nonatrophic Right Kidney	Corresponding Left Kidney
Av. wt. of kidney, gm.	5.3 (2.3-9.4)	25.6 (19.8-30.0)	11.5 (7.9-13.0)	25.1 (21.9-29.8)	21.3 (14.6-25.2)	24.1 (18.9-29.1)
Av. thickness of renal cortex, cm.	0.2	0.7	0.35	0.65	0.7	0.7
Av. thickness of cortex and medulla, cm.	1.0	2.0	1.5	2.0	1.9	2.1
Av. no. of glomeruli isolated from each kidney	225,000	614,375	481,250	450,000	700,000	750,000
Av. % of parietal capsules	9.0	22.2	22.6	21.0	44.0	68.0
% of tubular or cortical contaminants	None	None	None	8.3	None	5.5
Av. wt. of glomerulus, γ	0.22	0.27	0.28	0.3	0.27	0.33
Av. size of glomerulus without parietal capsule in stained mounts of isolated glomerular preparations, μ	144.5 × 112.0	234.0 × 189.0	191.5 × 141.3	—	—	—
Estimated % of acellular or parvicellular glomeruli	40	None	Negligible	None	None	None

TABLE 5.—*Titer of Antiserums to Twenty-Minute Sonically Vibrated Glomeruli Isolated from Kidneys Ten Weeks After Clamping Right Renal Artery*

	Markedly Atrophic Right Kidney		Corresponding Left Kidney		Mildly Atrophic Right Kidney *		Nonatrophic Right Kidney		Corresponding Left Kidney	
Amt. of Membrane Material Inoculated into Rabbits, Mg.	Est. Amt. Glomerular B. M.† (Estimated Nitrogen Content, Mg.)	No. Pos. Serums (No. Tested in Dogs)	Est. Amt. Glomerular B. M. (Estimated Nitrogen Content, Mg.)	No. Pos. Serums (No. Tested in Dogs)	Est. Amt. Glomerular B. M. (Estimated Nitrogen Content, Mg.)	No. Pos. Serums (No. Tested in Dogs)	Est. Amt. Glomerular B. M. (Estimated Nitrogen Content, Mg.)	No. Pos. Serums (No. Tested in Dogs)	Est. Amt. Glomerular B. M. (Estimated Nitrogen Content, Mg.)	No. Pos. Serums (No. Tested in Dogs)
2.5	2.2 (0.013)	1 (5)	1.5 (0.01)	2 (5)	1.9 (0.009)	1 (4)	1.36 (0.005)	0 (3)	0.94 (0.005)	0 (2)
5.0	4.4 (0.026)	3 (5)+‡	3.0 (0.02)	2 (5)	3.8 (0.017)	2 (3)+	2.7 (0.01)	0 (3)	—	—
10.0	8.8 (0.052)	3 (5)+	6.0 (0.04)	2 (5)	7.6 (0.036)	1 (2)	5.4 (0.02)	2 (3)+	3.7 (0.02)	2 (2)+
15.0	13.2 (0.078)	2 (3)+	9.0 (0.06)	2 (3)+	11.4 (0.052)	2 (2)+	7.9 (0.03)	0 (2)	—	—
Amt. of Supernatant Material Representing Cellular Components Predominantly Inoculated into Rabbits, Mg.										
50		1 (2)		0 (2)		—		0 (2)		0 (2)
125		1 (1)+		1 (1)+		—		2 (2)+		0 (2)

* Glomerular basement membrane from the corresponding left kidneys was not tested.
† B. M., basement membrane.
‡ +, positive titers.

were preserved, with no replacement whatsoever by fibrous tissue derived from the thin parietal capsule. The adjacent tubules likewise were acellular, with collapsed, folded, and wrinkled basement membranes. Few capillaries, fibroblasts, or histiocytes penetrated into this zone of necrosis, either from the renal capsule or

from the more viable, adjacent cortex. The non-necrotic areas were the seat of considerable atrophy (Fig. 6) with some calcific deposits in places. The glomeruli were mildly reduced in size, with moderately cellular-appearing tufts. The glomerular basement membranes appeared at times somewhat thickened, with some wrinkling and folding. For the most part, they appeared fairly normal. Red blood cells often filled the capillary lumens of these glomeruli. At times the tufts appeared to be irregularly filled with blood, or even to be bloodless. The atrophic tubules contained hyaline casts, which were PAS-positive. The medulla subtending areas of necrotic cortex presented collapsed, acellular tubular structures. The atrophic tubules in other areas contained casts. The main collecting ducts, even in necrotic zones, often presented a regenerated epithelium growing upward from the renal pelvis. Interstitial fibroblastic cells of the medulla were atrophic and distorted in the necrotic sectors but better preserved in the atrophic ones. Abundant iron deposits were demonstrable throughout the necrotic areas and in the viable cortex adjacent to these, where there were limited attempts at regeneration and repair.

The mildly atrophic kidneys came from four dogs (three female and one male), with preoperative weight of 15.2 lb. (12.0 to 18.5 lb.), or 6.9 kg. (5.4 to 8.4 kg.), and postoperative weight of 16.0 lb. (9.0 to 21.5 lb.), or 7.3 kg. (4.1 to 9.75 kg.). Here, too, the average blood pressure rose from 122.5 to 144 mm. Hg. These kidneys were a little smaller than normal and presented irregular areas of cortical atrophy, with, for the most part, well-preserved glomeruli and tubules (Fig. 7).

The nonatrophic kidneys came from five dogs (two female and three male), with a preoperative weight of 19.3 lb. (13.0 to 25.0 lb.), or 8.75 kg. (5.9 to 11.3 kg.), and a postoperative weight of 20.8 lb. (14.5 to 26.0 lb.), or 9.42 kg. (6.6 to 11.8 kg.). The average preoperative blood pressure was 131 mm. Hg and rose to 150 mm. Hg 10 weeks postoperatively. These kidneys appeared normal grossly and microscopically.

Data pertinent to these three sets of kidneys and the antigenic titers of the basement membranes of their isolated glomeruli are presented in Tables 4 and 5.

Associated with marked renal atrophy there were fewer glomeruli that were isolated from each kidney. These glomeruli were appreciably smaller, and 40% of them were acellular or parvicellular. Despite this, their basement membranes yielded serums with positive titer within the normal range of 4.4 mg. with 0.026 mg. of nitrogen, although one would have expected a greater per cent of positive serums at the next higher level of 8.8 mg. The supernatant, as in the 10-week hydronephrotic glomeruli, again showed a tendency toward increased fragility of the basement membrane, permitting on sonic vibration more of this material to be liberated in small particle form into the supernatant than was the case with normal glomerular basement membrane. Mild ischemia, as represented by the mildly atrophic kidneys, had little effect on the concentration of "nephrotoxic" antigen(s). Curiously enough, in the arterially clamped nonatrophic kidneys, while positive titer was reached at 5.4 mg. of basement membrane with 0.02 mg. of nitrogen, this was not maintained at the next higher level of titer, viz., 7.9 mg. with 0.03 mg. of nitrogen.

The basement membranes from the enlarged glomeruli of the hypertrophied kidneys contralateral to the markedly atrophic ones now more clearly demonstrated a decreased concentration of "nephrotoxic" antigen(s), with a positive titer at 9.0 mg. of basement membrane, or 0.06 mg. of basement membrane nitrogen, i. e., a twofold or threefold decrease in concentration. The serums derived from the base-

ment membranes of the nonhypertrophied glomeruli from the kidneys contralateral to the clamped nonatrophic ones yielded positive titers within the normal range.

Renal ischemia alone, thus, had little effect in varying the "nephrotoxic" antigenic concentration of glomerular basement membrane, although there was some tendency toward its reduction.

In addition the renal artery was clamped and the ureter on the same side ligated. Two such groups were studied, three to six weeks postoperatively. In one group, of 19 kidneys with marked renal necrosis and atrophy, the kidneys weighed 8.1 gm. (4 to 18 gm.). The pelves contained 8.3 cc. of urine (2 to 15 cc.). Only 213,947 glomeruli were isolated per kidney, and 50 to 55% of these were acellular or parvicellular. The isolated glomeruli on injection into rabbits yielded serums with a positive titer at the expected level for normal glomeruli with a corresponding percentage of parietal capsules of 15 mg., but failed to maintain it at levels of 20 and 25 mg. and only became positive again at 35 mg. The second group, of 12 kidneys, showed slighter degrees of necrosis but were more comparable in the degree of atrophy to the three-week hydronephrotic kidneys. The kidneys averaged 13.0 gm. in weight (7.8 to 19.6 gm.), and their pelves contained 13.0 cc. of urine (7.8 to 19.6 cc.). From each kidney, 253,750 glomeruli were isolated, and 5% of these were acellular or parvicellular. These glomeruli, despite a 19.5% parietal capsular content, failed to yield serums with a positive titer even at 25 mg., i. e., at the highest level employed in this instance.

The somewhat erratic behavior in antigenic content of glomerular basement membrane from arterially clamped nonureterally ligated kidneys and in that of glomeruli from clamped ureterally ligated and markedly atrophic and necrotic kidneys seemed to be due to a mechanism different from the more straightforward reduction in antigenic concentration observed with glomeruli from three-week hydronephrotic kidneys and those from the arterially clamped ureterally ligated ones with mild renal atrophy. It was clear, however, that the increased antigenic concentration of the glomeruli or their basement membranes derived from the 10-week hydronephrotic kidneys could not be explained by the same mechanisms applicable to either of the above.

Inferior Vena Cava Constriction.—In seven dogs (six female and one male), weighing 18.6 lb. (14.0 to 21.0 lb.), or 8.44 kg. (6.4 to 9.5 kg.), at the time of operation, the inferior vena cava was constricted above the level of the renal and adrenal veins, in the immediate subhepatic region. The vena cava was wrapped with dicetylphosphated Cellophane ¶ cut into a narrow strip 8 to 8.5 in. (20.3 to 21.6 cm.) long. An aluminum band was applied about the Cellophane wrapping to maintain the desired degree of constriction. A biopsy specimen of the left kidney was taken. During the ensuing 10 weeks neither peripheral edema nor ascites developed, and the weights of the dogs remained fairly constant, averaging 19.5 lb. (15.0 to 24.0 lb.), or 8.85 kg. (6.8 to 10.9 kg.), at the time they were killed. The veins of the hindlimbs and of the abdomen were prominent. Venous pressures taken from a vein of the hindleg rose from a preoperative level of 6.5 cm. (3.0 to 10.8 cm.) of 6% sodium citrate to 20.4 cm. (18.6 to 22.2 cm.) 2 weeks postoperatively, 17.7 cm. (14.6 to 21.2 cm.) 5 weeks postoperatively, and 18.3 cm. (14.0 to 23.2 cm.) 10 weeks postoperatively. Three of the dogs had proteinuria. The average diameter

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of the inferior vena cava prior to constriction was 1.05 cm. (0.85 to 1.2 cm.). It was reduced to 0.49 cm. (0.42 to 0.55 cm.) at the time of operation. At the time of autopsy the constricted portion of the vena cava measured 0.9 cm. in circumference (0.7 to 1.2 cm.), while caudal to the constriction the vena cava measured 2.2 cm. in circumference (1.4 to 3.0 cm.). There were no significant microscopic differences between the biopsy and autopsy renal specimens except for vascular congestion in the latter.

The inferior vena cava-constricted kidneys yielded, on the average, 546,428 glomeruli per kidney, with 19.4% parietal capsules and 3.3% tubular and cortical contaminants. Each glomerulus weighed 0.24 γ. The average size of the glomerulus with its parietal capsule, in paraffin-embedded sections of kidney, was 159.4 by

TABLE 6.—*Titer of Antiserums to Twenty-Minute Sonically Vibrated Glomeruli Isolated from Kidneys Ten Weeks After Inferior Vena Cava Constriction and from Comparable Control Kidneys*

	Inferior Vena Cava Constriction		Normal Controls					
Amt. of Membrane Material Inoculated into Rabbits, Mg.	Estimated Amt. of Glomerular Basement Membrane (Estimated Nitrogen Content, Mg.)	No. Positive Serums (No. Serums Tested in Dogs)	Estimated Amt. of Glomerular Basement Membrane (Estimated Nitrogen Content, Mg.)			No. Positive Serums (No. Serums Tested in Dogs)		
			A	B	C	A	B	C
2.5	1.8 (0.009)	5 (5)+*	1.6	2.4	1.3	0 (4)	0 (4)	1 (4)
5.0	3.6 (0.018)	5 (5)+	3.3	—	2.6	0 (4)	—	1 (4)
10.0	7.2 (0.036)	5 (5)+	—	—	5.2 (0.022)	—	—	2 (3)+
15.0	10.8 (0.054)	3 (3)+	—	—	7.8 (0.031)	—	—	3 (3)+
Amt. of Supernatant Material Representing Cellular Components Predominantly Inoculated into Rabbits, Mg.								
50		0 (2)				—	—	—
125		2 (2)+				—	—	—

* + = positive titers.

130.2 μ for the biopsy specimens and 154.8 by 129.6 μ for the 10-week vena cava-constricted ones. There was, therefore, no increase in size resulting from venous congestion over this period. The average size of the unencapsulated glomeruli from the vena cava-constricted kidneys in stained mounts of the isolated preparation was 215 by 165 μ, i. e., within the range for normal glomeruli isolated and measured in similar fashion in other experiments.

Three sets of isolated normal glomeruli were sonically vibrated and their basement membranes titered for "nephrotoxic" antigenic concentration as controls for the vibrated glomeruli from the vena cava-constricted kidneys. The control glomeruli were derived from kidneys which yielded 473,000 (A), 500,000 (B), and 588,889 (C) glomeruli per kidney with 29.3, 6.0, and 41.0% parietal capsules and 5.4, 0, and 15.1% contaminants, respectively. The glomerular weights were 0.25, 0.26, and 0.22 γ, respectively.

The results are listed in Table 6.

There was marked increase in "nephrotoxic" antigenic concentration of the glomerular basement membranes of the vena cava-constricted kidneys. All the serums were very strongly positive, including those at the lowest level tested, viz.,

1.8 mg. of basement membrane with only 0.009 mg. of nitrogen. The lowest limit of positive titer was not determined. By contrast, normal glomerular basement membranes yielded serums with positive titer within the expected range. Of 12 serums derived from 1.3 to 2.4 mg. of basement membrane, only 1 was positive, and of 8 at the next higher level, of 2.6 to 3.3 mg., only 1 was positive.

It would appear, therefore, that the increased concentration of "nephrotoxic" antigen(s) in the glomerular basement membrane of the 10-week hydronephrotic and the inferior vena cava-constricted kidneys was associated with a common underlying mechanism.

TABLE 7.—*Principal Qualities of Kidneys and Glomeruli Derived from Them Ten Weeks After Right Nephrectomy*

	Normal Right Kidney	Hypertrophied Left Kidney
Av. wt. of kidney, gm.	18.9 (13.0-26.3)	25.6 (17.1-37.5) representing an increase of 35.4%
No. of glomeruli isolated/kidney	508,333	525,000
% of parietal capsules	23	33
% of tubular or cortical contaminants	2.4	3.9
Av. wt. of glomerulus, γ	0.24	0.28
Av. size of glomerulus with parietal capsule in stained mounts of isolated glomerular preparations, μ	191.5 × 166.5	207.5 × 184

TABLE 8.—*Titer of Antiserums to Twenty-Minute Sonically Vibrated Glomeruli Isolated from Kidneys Ten Weeks After Right Nephrectomy*

	Normal Right Kidney		Hypertrophied Left Kidney	
Amt. of Membrane Material Inoculated into Rabbits, Mg.	Estimated Amt. of Glomerular Basement Membrane (Estimated Nitrogen Content, Mg.)	No. Positive Serums (No. Serums Tested in Dogs)	Estimated Amt. of Glomerular Basement Membrane (Estimated Nitrogen Content, Mg.)	No. Positive Serums (No. Serums Tested in Dogs)
2.5	1.4 (0.004)	0 (4)	1.0 (0.006)	0 (4)
5.0	2.8 (0.008)	0 (5)	2.0 (0.012)	0 (5)
10.0	5.6 (0.016)	3 (5)+*	4.0 (0.024)	1 (5)
15.0	8.4 (0.024)	1 (2)	6.0 (0.036)	1 (3)
Amt. of Supernatant Material Representing Cellular Components Predominantly Inoculated into Rabbits, Mg.				
50		0 (2)		1 (2)
125		2 (2)+		0 (2)

* + = positive titers.

Aside from the straightforward reduction in antigenic concentration observed with the slighter degrees of hydronephrosis (as exemplified by the three-week unilateral ureteral ligation and the mildly atrophic kidneys resulting from three- to six-week unilateral ureteral ligation combined with renal arterial clamping), there appeared to be a tendency toward a similar reduction in the case of glomeruli or their basement membranes derived from the compensatory hypertrophied kidneys. This reduction was indefinite and erratic in the case of the delayed hypertrophy in the 10-week hydronephrotic series, where it was felt that the enlarged hydronephrotic sac, either by pressure on the opposite renal vein or through reflex

mechanisms, might have interfered to some extent with the blood flow to the opposite kidney. In the case of the hypertrophied kidneys corresponding to the markedly atrophic and necrotic ones resulting from arterial clamping, there was, however, a more distinct reduction in antigenic concentration. It was, therefore, with that in mind that it was thought desirable to determine whether compensatory renal hypertrophy uninfluenced by the presence of one diseased kidney would more clearly indicate any variation in the antigenic concentration of the glomerular basement membrane.

Unilateral Nephrectomy.—Six dogs (five female and one male), weighing 14.9 lb. (11.0 to 16.5 lb.), or 6.76 kg. (5.0 to 7.5 kg.), had their right kidneys removed. Ten weeks later, at the time of killing, the dogs weighed 19.1 lb. (14.0 to 27.0 lb.), or 8.65 kg. (6.4 to 12.2 kg.). The pertinent data are recorded in Table 7.

The rabbit antiserums to the sonically vibrated glomerular sediments and supernatants titered as shown in Table 8.

These results indicate that the basement membrane of normal glomeruli yielded positive serums within the expected range of 5 mg. and 0.02 mg. of nitrogen. The serums derived from the basement membranes of the hypertrophied glomeruli, however, failed to titer positively at 6 mg., or at 0.04 mg. of nitrogen, indicating a loss in antigenic content of the specific "nephrotoxin." The serums from the supernatants of the sonically vibrated normal glomeruli titered positively within the expected range of 125 mg. The fact that the serums of the supernatants from the hypertrophied glomeruli failed to yield a positive titer at 125 mg. may indicate either the usual expected variation or, possibly, a decreased fragility of the basement membranes.

Unilateral Nephrectomy and Partial Corticectomy of the Contralateral Kidney.—In order to place the maximum amount of work upon the glomeruli, the left kidney was removed. At the same time, in order to avoid the complications of seepage of urine from the renal pelvis when a heminephrectomy is performed, the cortex alone was removed from the lower half of the right kidney. A ligature was placed around the right renal vascular pedicle and was drawn taut to control bleeding. The cortex was then incised and removed with forceps and small curved scissors right down to the distinct red corticomedullary junction or to the gray medulla. The cortical strips were collected and weighed. The denuded surfaces were covered with a thin layer of Gelfoam. The ligature on the pedicle was slowly released. Oozing through the Gelfoam promptly ceased, and the kidney was gently replaced in its bed. Eleven female dogs were so operated upon. The average weight of these dogs at the time of operation was 14.7 lb. (12.0 to 19.0 lb.), or 6.68 kg. (5.4 to 8.6 kg.). Ten weeks later, at the time of killing, these dogs weighed 16.0 lb. (12.0 to 24.5 lb.), or 7.3 kg. (5.4 to 11.1 kg.).

During this 10-week interval the following observations were made: Two weeks postoperatively the average daily urinary output of seven dogs was 365 cc. (310 to 505 cc.), with a specific gravity of 1.023. Faint amounts of protein were detectable in the urines of three dogs. The average femoral blood pressure of 10 dogs was 134.5 mm. Hg (129 to 140 mm.). Six weeks postoperatively the daily urinary output of five dogs was 290 cc. (140 to 450 cc.), with a specific gravity of 1.023. Three dogs had a faint proteinuria. The blood pressure of 10 dogs at this time averaged 131.2 mm. Hg (128 to 136 mm.), and the serum nonprotein nitrogen in four dogs was 144 mg. per 100 cc. (120 to 162 mg.). The values in these four dogs dropped

to 102 mg. per 100 cc. of nonprotein nitrogen four weeks later. Eight weeks postoperatively the blood pressure of nine dogs was 131 mm. Hg (128 to 134 mm.). At the end of 10 weeks the nonprotein nitrogen determined in 10 dogs averaged 116 mg. per 100 cc. (90 to 127 mg.), and the blood pressure in 11 dogs averaged 136 mm.

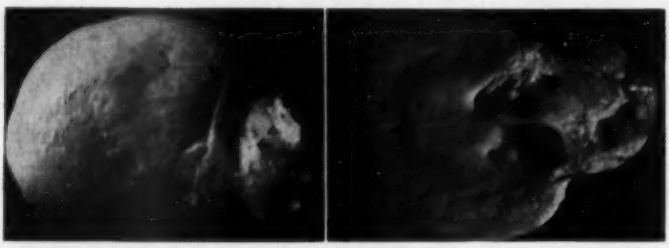

Fig. 8.—External and sectioned aspects of partially corticectomized kidney 10 weeks after unilateral nephrectomy and partial corticectomy of contralateral kidney. Note that the corticectomized sector appears as an appendage to the remaining hypertrophied sector of kidney.

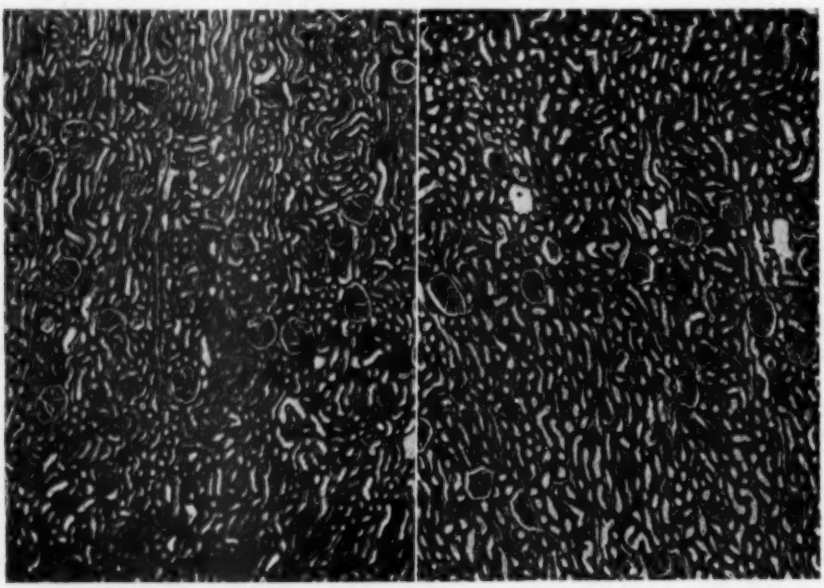

Fig. 9.—Microscopic appearance of normal kidney to the left, and that of the hypertrophied sector of the partially corticectomized kidney shown in Figure 8 to the right. Note the more widely spaced glomeruli and greater abundance of tubular structures in the hypertrophied cortex. Hematoxylin and eosin; × 42.

Hg (122 to 143 mm.). There was, therefore, a good urinary output of high specific gravity and no elevation in blood pressure but appreciable azotemia.

The appearance of the partially corticectomized kidney 10 weeks postoperatively is demonstrated in Figure 8. The corticectomized sector shrank, forming an append-

age to the hypertrophied cranial portion of the kidney. Grossly, the cortex was absent, and a thin fibrous scar formed a smooth pseudocapsule. Microscopically, only a few juxtamedullary glomeruli were identified here and there in the outer, scarred area. The medulla in this corticectomized region was reduced in thickness. There were collapsed tubules lined by atrophic cells, with condensation of the peri-

TABLE 9.—*Principal Qualities of Kidneys and Glomeruli Derived from Them Ten Weeks After Left Nephrectomy and Partial Corticectomy of Right Kidney*

	Normal Left Kidney	Partially Corticectomized Hypertrophied Right Kidney
Av. wt. of kidney, gm.	18 (11.5-26.1)	18.7 (12.5-33.4)
Av. amount of cortex removed, gm.	—	5.9 (2.9-10.0) Estimated 54.6% of original amount of cortical substance
No. of glomeruli isolated/kidney	613,636	221,428
% of parietal capsules	37	7
% of tubular or cortical contaminants	1.8	None
Av. wt. of glomerulus, γ	0.27	0.36
Av. size of glomerulus without parietal capsule in stained mounts of isolated glomerular preparations, μ	222.5 × 175.5	237.5 × 183.5

TABLE 10.—*Titer of Antiserums to Twenty-Minute Sonically Vibrated Glomeruli Isolated from Kidneys Ten Weeks After Left Nephrectomy and Partial Corticectomy of Right Kidney*

	Normal Left Kidney		Partially Corticectomized Hypertrophied Right Kidney	
Amt. of Membrane Material Inoculated into Rabbits, Mg.	Estimated Amt. of Glomerular Basement Membrane (Estimated Nitrogen Content, Mg.)	No. Positive Serums (No. Serums Tested in Dogs)	Estimated Amt. of Glomerular Basement Membrane (Estimated Nitrogen Content, Mg.)	No. Positive Serums (No. Serums Tested in Dogs)
2.5	1.35 (0.005)	0 (4)	2.35 (0.011)	0 (5)
5.0	2.70 (0.009)	0 (4)	4.70 (0.022)	0 (5)
10.0	5.40 (0.018)	4 (4)+*	9.40 (0.044)	3 (5)+
15.0	8.10 (0.028)	3 (3)+	14.10 (0.066)	3 (3)+
Amt. of Supernatant Material Representing Cellular Components Predominantly Inoculated into Rabbits, Mg.				
50		0 (2)		0 (2)
125		1 (1)+		1 (1)+

* + = positive titers.

tubular connective tissue. The main collecting ducts were fairly well preserved. PAS-positive casts were present in the atrophic tubules at the corticomedullary junction. There was PAS-positive material in the cells of the collecting ducts and pelvic epithelium. Iron deposits occurred in the operative zone and in the sclerosed medulla. The noncorticectomized portion of the kidney revealed hyperplasia of the convoluted tubules in terms of increased length and size (Fig. 9). The lumens of these tubules were widened. Afferent and efferent arterioles were wider and more prominent than in the normal kidney. There was little evident change in thickness of the glomerular basement membrane.

The data relevant to the kidneys of this experiment are recorded in Table 9. The renal cortex, on the average, represents 60% of the total weight of the normal kidney. On the assumption that the right and the left kidney weighed about the same at the time of operation, the percentage of cortical material removed could be roughly estimated. Approximately 5.9 gm., with a range of 2.9 to 10.0 gm., of cortex were removed, representing 54.6% of the total amount of cortical substance of the remaining kidney.

The hypertrophied sector of the partially corticectomized kidney had a cortex averaging 0.8 cm. in thickness, i. e., 0.1 to 0.2 cm. thicker than the normal cortex. The glomeruli were only modestly enlarged but appreciably heavier. The yield of glomeruli, although less than the expected half, represents, however, only 18% of the estimated total number of glomeruli in the previously existent intact two kidneys. It is remarkable that with such a small percentage of total glomeruli these dogs were able to eliminate an abundant volume of urine of high specific gravity. This amount, however, was inadequate to maintain a normal blood nonprotein nitrogen. These dogs showed no obvious evidences of uremia and, in fact, gained weight during the postoperative period.

The rabbit antiserums to the sonically vibrated sediments and supernatants of the isolated glomeruli of these kidneys titered as shown in Table 10.

Normal glomerular basement membrane again yielded serums with positive titer within the expected range of 5 mg. with 0.02 mg. of nitrogen. The basement membrane of the hypertrophied glomeruli, however, showed distinct reduction in antigenicity, yielding serums with positive titer between 9.4 and 14.1 mg., or between 0.04 and 0.07 mg. of nitrogen. This represented a decrease in concentration of the "nephrotoxic" antigen(s) of from twofold to threefold. The supernatants in both preparations behaved in the expected fashion.

It seemed safe to conclude that compensatory renal hypertrophy was associated with reduction in "nephrotoxic" antigenic concentration of the glomerular basement membrane.

In order to establish some basis for the decreased antigenic concentration of glomerular basement membrane under certain circumstances, an increase under others, and, again, slight and erratic variations under a third set of conditions, it was felt that as background material it was essential that we determine whether any such variations existed in developing glomeruli during the formative stage of the kidney. If these variations under induced experimental conditions were due to altered blood flow and pressure relationships as they relate to tension or lateral forces exerted upon the basement membrane of the unsupported glomerular capillaries, then the fetal hemodynamic flow of blood, with its lower arterial pressure and diminished volume, should reflect a difference in the concentration of "nephrotoxic" antigen(s) within the fetal glomerular basement membrane.

In order to simplify the problem of procurement of material, it was decided to use kidneys from newly born pups, generally obtained within a few hours after birth. As it turned out, for even these limited experiments, 780 neonatal kidneys were required.

Observations on Neonatal Kidneys.—The neonatal kidney is admirably adapted to the study of the concentration of "nephrotoxic" antigen(s) as it pertains to growth and development of this organ. Its cortex can conveniently be divided into three zones (Fig. 10). There is a neoformative layer, approximately 0.5 mm. thick,

in which there are developing and newly formed glomeruli surrounded by embryonic tubules. This zone rapidly decreases in size and disappears within 10 days to two weeks postnatally. Next to it there is a midzone, about 0.5 mm. in thickness, in which the glomeruli gradually increase in size. Their tufts become more apparent, and the capillaries are a little wider and better filled with blood. The glomeruli are still rather cellular in appearance, however, largely as a result of cells between the capillary loops. The glomerular basement membrane is thinner than in the maturer glomeruli of the juxtamedullary zone. The visceral epithelial cells are now a little farther apart and are flatter as compared with their close-set, tall, columnar quality and little cytoplasm in the immature glomeruli in the neoformative zone. In this midzone the labyrinth about the glomeruli is now made up of differentiated tubules. The 0.5 mm. or so of the juxtamedullary portion of the cortex includes maturer glomeruli, approaching adult size and form in the deepest tier. These are more widely dispersed, in a more abundant labyrinth. There are approximately 7 or 8

TABLE 11.—*Titer of Antiserums to Cortical Shavings from Neoformative, Midzone, and Juxtamedullary Layers Respectively of Neonatal Kidney*

Amt. of Material Inoculated into Rabbits, Mg.	Neoformative Layer, No. Positive Serums (No. Serums Tested in Dogs)	Midzone Layer, No. Positive Serums (No. Serums Tested in Dogs)	Juxtamedullary Layer, No. Positive Serums (No. Serums Tested in Dogs)
75-100	—	2 (8)	0 (3)
150	—	2 (7)	2 (6)
200-250	—	4 (5)+*	1 (5)
250-300	0 (4)	3 (4)+	—
300-350	—	2 (5)	3 (4)+
500 plus	1 (5)	—	9 (13)+
1,000	0 (7)	—	—
1,500-2,500	2 (3)+	—	—

* + = positive titers.

tiers of glomeruli in the whole neonatal cortex, as compared with 9 or 10 in the adult, with 2 or 3 tiers in each of the above zones. There are approximately three times as many glomeruli in the neoformative as in the juxtamedullary cortex per unit area and about twice as many in the midzone as in the juxamedullary portion of the cortex. The formed immature glomeruli in the neoformative zone measure 69.6 by 43.6 μ in stained sections, excluding the capsular space, whereas in the adult kidney the glomeruli next the cortex corticis measure 116.0 by 97.8 μ. In the same way, in the midzone the glomeruli measure 84.4 by 55.0 μ, while in a corresponding site in the adult cortex they measure 118.0 by 99.8 μ. The largest juxtamedullary glomeruli in the neonatal cortex measure 118.6 by 90.8 μ, while those in the adult cortex measure 140.2 by 118.2 μ.

In order to determine whether there were any detectable differences in the antigenic concentration of the glomeruli in these different layers, the shavings from these three different zones were tested after being duly processed, as detailed in the procedure. On the average the neoformative layer yielded 53.7 mg. per kidney (26.7 to 76.2 mg.), the midzone 36.2 mg. (23.7 to 49.6 mg.), and the juxtamedullary zone 169.6 mg. (96.1 to 293.8 mg.). The results are tabulated in Table 11.

The neoformative layer contained about three times the number of glomeruli as the juxtamedullary portion of the cortex, although these glomeruli were about half, or less, the size of the maturer glomeruli. Furthermore, since these were contained

within an amount of cortex one-third that of the juxtamedullary zone, it is clear that for any unit amount of material injected, there would be nine times the number of neoformative glomeruli, or, roughly, four times the amount of glomerular material in terms of equivalent size as compared with that in the juxtamedullary cortex. Despite this, the neoformative layer yielded serums with positive titer between 1.5 and 2.5 gm., while that of the juxtamedullary layer did so at 300 to 350 mg. It is certain that, even at that, increasing numbers of glomeruli from the midzone were included with the increasing amounts of neoformative cortex used for injection.

The concentration of "nephrotoxic" antigen(s) in the formative and immature glomeruli of the neoformative zone must be very low indeed.

By contrast, the glomeruli of the midzone, now presenting qualities compatible with function in terms of serving as filtering units and with maturer cortex about them, contained an appreciable amount of "nephrotoxic" antigen(s), but less than that in the juxtamedullary zone. The midzone contained roughly twice the number of glomeruli in a fourth or fifth of the amount of cortex as did the juxtamedullary zone. These glomeruli were about two-thirds or so the size of the larger juxtamedullary glomeruli. For any given unit amount there were, roughly, in terms of equivalent size, six times as much glomerular material in the midzone as in the juxtamedullary zone. Since the former yielded serums with positive titer at 200 to 250 mg. and the latter at 300 to 350 mg., it seems reasonable to assume that the glomeruli of the midzone contained a lower concentration of antigen(s).

It would appear, therefore, that the glomerulus acquires an appreciable concentration of "nephrotoxic" antigen when it assumes the appearance of a functional filtering unit, i. e., at a time when intracapillary blood pressure and flow are presumably such as to permit these functions. Furthermore, the concentration increases as the glomerulus enlarges and matures—in other words, where pressure and flow are greater.

Attempts were then made to titer the amount of antigen(s) in whole glomeruli isolated from these portions of the neonatal cortex in order to compare them with adult glomeruli. It was found impossible to isolate glomeruli from the neoformative layer, since the embryonic tubular structures came through the sieve with facility equal to that of the glomeruli. The same was also true for the thin shavings of the midzone. It was only by cutting away midzone and juxtamedullary cortex, or the latter alone, from the medulla, and thus obtaining larger continuous pieces, that one could attempt to isolate glomeruli. Even at that, it proved to be a formidable task to obtain clean preparations, and the yields were exceedingly small. We had, therefore, to be content to use preparations with a higher percentage of tiny tubular and bits of cortical contaminants than was customary with such preparations from adult kidneys.

The five samples of glomeruli obtained from the combined midzone and juxtamedullary zone contained on the average 17.6% contaminants, while 14.9% (6 to 29%) of the glomeruli retained their parietal capsules. The average weight of the glomerulus was 0.07 γ (0.03 to 0.09 γ), i. e., approximately one-third or one-fourth that of an adult glomerulus as determined in the usual preparations with varied proportions of parietal capsules. Five samples of glomeruli isolated from the juxtamedullary layer alone contained an average of 15.4% contaminants, and

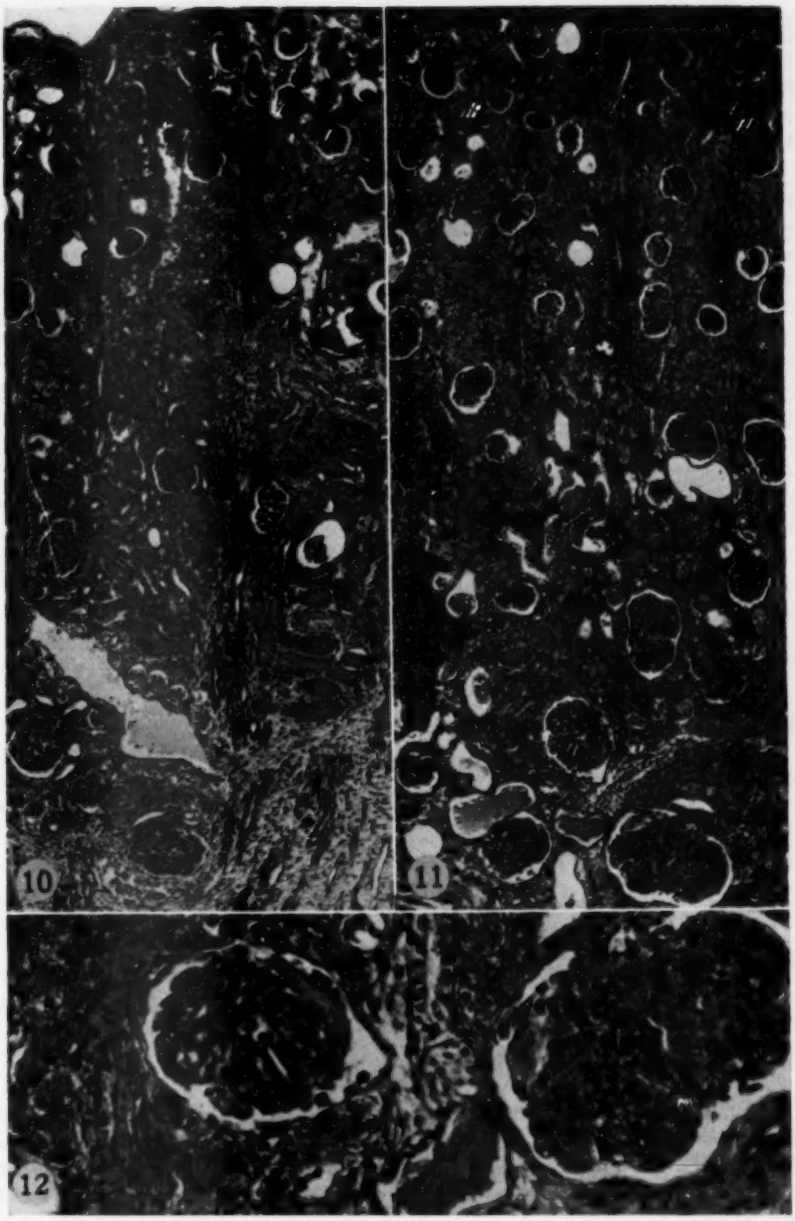

Fig. 10 (Upper left).—Microscopic section of neonatal cortex in a normal pup a few hours after birth. Note the neoformative layer in the upper third of the photograph. The midzone occupies the middle third, and the juxtamedullary zone, the lower third. Hematoxylin and eosin; × 100.

Fig. 11 (Upper right).—Five-day-old pup inoculated intravenously 24 hours after birth with 5 cc. of lyophilized "nephrotoxic" serum, the equivalent of 20 cc. of serum. Microscopic appearance of the cortex of the kidneys showing enlargement and increased cellularity of the glomeruli in the juxtamedullary zone, with slight to mild involvement of the lower tiers of glomeruli in the midzone. Hematoxylin and eosin; × 100.

Fig. 12.—Enlargement of the juxtamedullary glomeruli in Figure 11 showing some fibrinous and hemorrhagic exudate over the medial aspects of the two glomeruli and the avascular and cellular appearance of the tufts. Note hemorrhagic casts in the contiguous tubules. Hematoxylin and eosin; × 270.

11.1% (6 to 17%) of the glomeruli retained their parietal capsules. The average weight of a glomerulus was 0.08 γ (0.07 to 0.11 γ), with an average volume of 35 × 10^3 cu. μ, i. e., approximately 1/10 that of the adult glomerulus.

Since there appeared to be no significant difference in the titers of the isolated glomeruli from the combined midzone and juxtamedullary zone and from the juxtamedullary zone alone, although this absence was due in part to a smaller number of trials than was desirable, the combined results are presented in Table 12.

TABLE 12.—*Titer of Antiserums to Glomeruli Isolated from Midzone and Juxtamedullary Zone of Neonatal Cortex*

Amt. of Glomerular Material Inoculated into Rabbits, Mg.	No. Positive Serums (No. Serums Tested in Dogs)
15	1 (6)
20-25	0 (7)
35	3 (6)
40-55	2 (9)
70-75	5 (7)+*
80-150	6 (8)+

* + = positive titers.

TABLE 13.—*Titer of Antiserums to Twenty-Minute Sonically Vibrated Glomeruli Isolated from Mid- and Juxtamedullary and Juxtamedullary Cortical Layers of the Neonatal Kidney Respectively*

Amt. of Membrane Material Inoculated into Rabbits, Mg.	Mid- and Juxtamedullary Zones, No. Positive Serums (No. Serums Tested in Dogs)	Juxtamedullary Zone, No. Positive Serums (No. Serums Tested in Dogs)
2.5	—	0 (3)
5.0	0 (4)	0 (10)
10.0	0 (5)	3 (8)
15.0	1 (3)	—
Amt. of Supernatant Material Representing Cellular Components Predominantly Inoculated into Rabbits, Mg.		
35	2 (2)+*	0 (3)
75	1 (2)	1 (1)+

* + = positive titers.

On a weight basis, the level of serums with positive titer at 70 to 75 mg. is considerably greater than would be expected if these immature glomeruli had the same concentration of "nephrotoxic" antigen(s) as the adult ones. Adult glomeruli with such a low parietal capsular content yield serums with positive titer at a level of 15 to 20 mg.

The immature glomeruli have a greater degree of cellularity than the adult and, accordingly, have a higher density, as indicated by their weight and volume relationships. Chemical analyses of the isolated neonatal glomeruli likewise indicated the higher proportion of cellular elements by a somewhat increased content of phosphorus and DNA as compared with that of the adult. The values on a fat-free dry weight basis were 1.3% of phosphorus, 13.1% of nitrogen, and 5.12% of DNA

(compare with figures in Table 1). It was for this reason that the neonatal glomeruli were sonically vibrated for 20 minutes and the sediment representing basement membranes, in great part devoid of cellular constituents, was titered. The results are given in Table 13.

Owing to small yields, particularly from the juxtamedullary cortex, it was difficult to obtain adequate material to test higher levels of titer. Nevertheless, while for glomeruli with low parietal capsular content in the adult the positive titer of serums prepared against its basement membrane (uncorrected for its parietal capsular content) is 5 to 10 mg., the less mature glomeruli included in the midzone and juxtamedullary zone combined failed to yield serums with positive titer even at 15 mg. The supernatant again presented features indicating increased fragility of the basement membrane of these immature glomeruli.

The maturer glomeruli from the juxtamedullary zone failed to yield serums with positive titer at 10 mg. but might have at 15 mg. It is evident that there is a somewhat decreased concentration of "nephrotoxic" antigen(s) in the maturer glomeruli of the neonatal kidney than in the kidney of the adult. There was little indication that the basement membrane, which is thicker in the maturer glomeruli, showed any tendency toward increased fragility.

In order to establish these views concerning the rise in antigenic concentration with presumed glomerular function and the increasing concentration thereafter as the glomerulus matures, the distribution of glomerular lesions was studied in pups injected intravenously with rabbit anticanine glomerular "nephrotoxic" serum. At the same time appropriate controls were injected with isotonic saline or normal rabbit serum. To assure ourselves that adequate nephrotoxic antibodies would reach all levels of the glomeruli in the neonatal cortex, a small amount of Evans blue was injected intracardially into pups. They were killed immediately thereafter. The kidneys were fixed and sectioned. The dye was found to be distributed throughout the cortex, including the neoformative zone, indicating a rapid and diffuse flow of blood. Likewise, Fergusson [19] has recently shown that India ink injected into the aorta of neonatal kittens and rabbits will immediately flush the nephrogenic cortex and that traces of the ink can be discerned even in the S-shaped developing glomeruli in that zone of cortex. For this reason, varied amounts of antiserum were injected, including lyophilized serum so diluted that the 5 cc. injected actually represented 20 cc. of "nephrotoxic" serum. This amount was used in order to assure an excess of antibodies over and above that which might be taken up by the maturer glomeruli with a greater flow of blood through them or that which might be bound or lost in extrarenal sites.

Of seven pups one day old, weighing 334 gm., four were injected with 0.85 cc. of "nephrotoxic" serum and three with 5 cc. of lyophilized "nephrotoxic" serum. They were killed two to seven days later, weighing on the average 283 gm. All developed glomerulonephritis, with loss of weight and a 1+ to 2+ proteinuria. The glomeruli involved were those in the first three juxtamedullary tiers, with little or no involvement of the remaining tiers in the midzone. The glomeruli in the neoformative zone were never affected. The involved glomeruli presented considerable or some degree of enlargement, with endothelial and, to some extent, visceral epithelial hypertrophy and proliferation, resulting in decreased vascularity of the tufts. There was little exudate in the capsular spaces, but in the immediate juxtamedullary glomeruli there were occasionally necroses and thromboses of the tufts.

The adjacent tubules contained hemorrhagic casts (Fig. 11). By contrast with "nephrotoxic" serum glomerulonephritis in the adult dog, there was (1) the limited distribution of lesions to the juxtamedullary glomeruli, so that the external surfaces of the kidneys at no time showed petechial hemorrhages, (2) limited exudative and hemorrhagic phenomena, principally in the largest juxtamedullary glomeruli, and (3) the much earlier onset of the disease, within 48 hours after injection, by contrast with the five- to seven-day period in the adult animal. This short latent period is more in keeping with that seen in the rat, where the nephritic process sets in shortly after injection of rabbit antirat kidney serum, as demonstrated by Masugi,[20] Smadel and Farr,[21] Heymann and Hackel,[22] and Lippman and associates.[23] The control animals showed no such lesions.

Four pups, 2 days old, weighing 278 gm., were inoculated with 4.2 cc. of "nephrotoxic" serum. They were killed five to seven days later, weighing 273 gm. Here, too, the juxtamedullary three or four tiers of glomeruli were affected, while the outer four or five tiers were uninvolved. All pups had proteinuria. The serum N. P. N. values were elevated from a normal of about 31 mg. per 100 cc. to 45 up to 60 mg. per 100 cc. The plasma proteins dropped from 4 gm. per 100 cc. to 3.35 to 3.75 gm. per 100 cc.

Thirteen pups, 3 days old, weighing 340 gm. (180 to 490 gm.), were inoculated with amounts of "nephrotoxic" serum varying from 0.75 to 2.5 cc., including 4 animals inoculated with 5 cc. of lyophilized "nephrotoxic" serum. These were killed four to eight days later, weighing 325 gm. (150 to 510 gm.). Nine control animals, with a starting weight of 288 gm. (150 to 400 gm.), during the same experimental period gained weight up to an average of 420 gm. (120 to 600 gm.), except for one animal, whose weight dropped from 150 to 120 gm. Here, again, only the juxtamedullary four tiers of glomeruli were principally affected in the experimental animals, in the same manner as described for the one-day-old pups. Most of the experimental animals developed proteinuria, and there was an elevation in the N. P. N. in the few for which this value was determined.

On the other hand, six pups, 36 days old, weighing 1,400 gm. and inoculated with 6.3 to 10.5 cc. of "nephrotoxic" serum in two consecutive daily injections, were observed to have developed (when killed on the seventh day) diffuse glomerulonephritis involving all glomeruli, as in the adult. Their average weight at that time was 1,660 gm. The renal cortex measured about 3 mm. in thickness, as determined in paraffin sections with a cortex corticis about 0.25 mm. in thickness. The same diffuse glomerulonephritic changes were observed in three young dogs, 71 days of age, weighing 4.0 to 5.3 lb. (1.8 to 2.42 kg.), seven days after intravenous inoculation with 20 cc. of "nephrotoxic" serum. The renal cortex at this age in fixed sections approached that of the adult, measuring 4.0 to 4.8 mm., with a cortex corticis of 0.4 to 0.5 mm.

In none of these animals were there visceral lesions attributable to the "nephrotoxic" serum, except those in the kidney.

In evaluating these results, it is essential to bear in mind that the "nephrotoxic" antibodies are largely removed from the circulation within 15 to 20 minutes after injection, as shown through different experimental procedures by Sarre and Wirtz,* Kay.[26] Pressman and his co-workers,† Lippman and associates,[30] and, recently,

* References 24 and 25.
† References 27 and 29.

Röther.[31] Most of these antibodies are taken up by the glomeruli. Furthermore, it requires an active flow of blood through the glomeruli if they are to absorb an adequate number of antibodies. This requirement was clearly shown by Sarre and Wirtz*; for when the artery of one kidney was clamped for 15 minutes and "nephrotoxic" serum injected at the time the clamp was applied, the clamped kidney failed to develop glomerulonephritis but the opposite kidney did. In other words, the normal kidney had removed most of the antibodies, leaving too few to affect the clamped kidney, after the clamp had been removed. Nevertheless, with the precautions taken here to supply an excess of "nephrotoxic" antibodies, even though the greater volume of blood flow is through the juxtamedullary glomeruli, the results appear to be confirmatory of those obtained by directly titering the antigenic content of the neonatal glomeruli. There is good evidence, therefore, that the concentration of "nephrotoxic" antigen(s) increases with maturation of the glomeruli; i. e., there is an increasing number of antigenic sites within the glomerular basement membrane to which circulating antibodies can become attached.

A similar approach was tried in adult dogs in an attempt to confirm the findings of variation in "nephrotoxic" antigenic concentration of glomerular basement membrane under different experimental conditions. This was attempted in animals with unilateral hydronephrosis, only to meet with the problem of adequate circulation through the hydronephrotic kidney, as indicated above. Thus, three three-week unilateral hydronephrotic animals were inoculated intravenously with rabbit anti-canine glomerular basement membrane serum at the rate of 1.5 cc. per pound. They were killed seven days later. The hydronephrotic kidneys failed to demonstrate any glomerulonephritic lesions grossly (Fig. 1). Microscopically, there were no or minimal glomerular lesions. The nonhydronephrotic kidneys presented classic gross and microscopic features of hemorrhagic glomerulonephritis. With a view to permitting the maximum number of antibodies to reach the hydronephrotic kidney, the vascular pedicle of the opposite, unaffected kidney was clamped in two 3-week and one 10-week unilateral hydronephrotic animals. The "nephrotoxic" serum was administered intravenously, as above, while the animals were under anesthesia. The clamp was removed at the end of 30 minutes. The animals were examined seven days later. In general, the hydronephrotic kidneys again showed few or no glomerular lesions, but in the less atrophic areas there were mild proliferative changes or even, at times, some capsular exudate, while the nonhydronephrotic kidneys showed classic glomerulonephritis in two animals and mild diffuse glomerulonephritis in one (Fig. 3). On the assumption that the raised intrinsic pressure within the kidney might interfere with the proliferative and exudative responses even though the glomeruli had absorbed an adequate number of antibodies, the contents of the hydronephrotic kidney were removed in one 10-week unilateral hydronephrotic animal. The renal pedicle of the opposite kidney was clamped and "nephrotoxic" serum injected intravenously. The clamp was removed after 30 minutes. Seven days later the hydronephrotic sac had partially refilled. The glomeruli in the more markedly atrophic areas were unaffected. In the less atrophic zones there were some proliferative glomerular changes with, in some instances, necrosis of loops and some capsular exudate. The opposite kidney showed classic glomerulonephritis. Here, too, in all these animals there were no visceral lesions except in the kidneys.

It was clear, therefore, that no matter whether the glomerular basement membrane had apparently acquired more "nephrotoxic" antigen(s), as in the 10-week hydronephrotic kidney, or had temporarily lost some, as in the 3-week hydronephrotic one, unless there was an adequate volume of blood flow through the glomeruli, there would be no way of demonstrating these variations by their ability to react to varying concentrations of "nephrotoxic" antibodies. For those kidneys with unimpeded circulation, as in compensatory hypertrophy following unilateral nephrectomy, with or without combined contralateral corticectomy, or in the group with inferior vena cava constriction, it was felt that it was not practical to work with dogs in order to elicit these differences in antigenic concentration. This must await trials in smaller experimental animals, such as rats.

COMMENT

There is substantial evidence from the present findings that the concentration of "nephrotoxic" antigen(s) in the glomerular basement membrane is subject to quantitative variation. It would be most logical to attempt to explain these variations on the basis of physical and chemical alterations within the basement membrane secondary to alterations in pressure and flow of blood within the glomerular capillaries. The glomerular capillaries are unique, since they are virtually unsupported structures projected into a capsular space. They lack the connective tissue and parenchymal supports common to capillaries elsewhere. Furthermore, intraglomerular capillary blood pressure exceeds that within capillaries elsewhere, being double, or more, that given for capillaries in tissues.

Burton [32] has recently studied the tension which develops within the walls of larger blood vessels, with variation in blood pressure and volume flow. He has found that Laplace's Law, relating tension directly to the product of pressure within and the radius of a tube conducting fluid, pertains, in the main, to these larger blood vessels but is not applicable to ordinary capillaries. It may be different in the case of glomerular capillaries, however. Since the variation in radius of the glomerular capillaries would be very small, the tension within the basement membrane would vary directly with the pressure within the capillaries.

If the results of the present experiments are viewed in the light of tension developed within the glomerular basement membrane and intrinsic physical and chemical adjustments thereto, it would appear that increased concentration of antigen is associated with increased tension. Thus, with constriction of the inferior vena cava the elevated renal venous pressure should logically lead to an increase in intraglomerular capillary pressure and to a certain amount of slowing of blood flow, resulting in increased lateral pressure. In the case of the 10-week hydronephrotic kidney not only is there the element of marked disturbance in venous return, but, in addition, the high intrinsic pressure developed within the renal substance is enough in itself to place the membranes on stretch. This is evident if the large hydronephrotic kidney is fixed prior to release of the fluid. Under those circumstances histologic sections clearly demonstrate elongation and distortion of the glomeruli, as well as of the tubules.

By the same token, decreased tension within the glomerular basement membrane would be associated with decreased concentration of "nephrotoxic" antigen(s). In the case of the neonatal glomeruli it is logical to assume that the increasing size of

the glomeruli from neoformative to juxtamedullary zone is associated with increased volume of blood flow and, probably, intrinsic pressure which, even in the most mature ones next to the medulla, are in all likelihood less than those in the adult. This is an instance in which tension placed upon the membrane can to some extent be correlated with its thickness, since the glomerular basement membrane of the more immature glomeruli is clearly thinner than that of the maturer ones. In the adult, however, except for the apparent thickening and folding of the membrane in the atrophic glomeruli of the 10-week hydronephrotic kidney and for the somewhat similar change in some of the glomeruli in the markedly ischemic kidneys, there was no correlation between concentration of "nephrotoxic" antigen(s) and thickness of the basement membranes. Minor changes of that type in the absence of glomerular atrophy could, however, be readily missed, since it is difficult to measure the thickness of the membrane accurately. It has been estimated that the blood flow in the compensatory hypertrophied kidney following unilateral nephrectomy in dog and in man may be increased to the extent of 70 to 100% of what it was formerly.[83] This large increase in blood flow through the glomeruli might very well be associated with a sharper drop in pressure from afferent to efferent arterioles and with increased velocity of flow. These factors are operative in reducing lateral pressure upon the walls of blood vessels. For the three-week hydronephrotic kidneys the reduction in concentration can, again, be explained on a similar principle. In the early development of hydronephrosis there is increasing intrarenal pressure to the point where it almost becomes equivalent to the difference between intraglomerular capillary pressure and the osmotic pressure of plasma protein, thus reducing the filtration pressure. It is clear that, if tension on the basement membrane is in great part due to the differences between intraglomerular capillary blood pressure and capsular or intrarenal pressure, elevating the latter will serve to buttress, or support, the capillaries and thus diminish tension within the basement membrane, even though as a result of interference, particularly with venous flow, there may be increased intraglomerular pressure. As, however, more fluid accumulates in the later phases of hydronephrosis, the actual intrarenal pressure is such as to stretch and deform the glomeruli at a time when ischemia is not so profound as in the clamped renal arterial experiments, to be discussed below.

The minor variations in antigenic concentration with ischemia induced by clamping the renal artery have not been as easy to explain. With mild, irregular atrophy and in those instances with little atrophy of the kidneys, there is no reason to doubt that there has been, at least within a 10-week period, readjustment of circulation so that there has been little alteration in intraglomerular hemodynamics from that of the normal. With intense ischemia one must assume that, where the glomeruli have undergone necrosis with loss of their cellular elements but maintenance of their basement membranes, these membranes have retained their original fixed amount of antigen(s). This is compatible with the view that it requires an adequate blood flow for metabolic changes of any order to take place in the membrane. In the absence of such blood flow neither an increase nor a decrease in antigen(s) would be expected. However, the remaining, somewhat atrophic, glomeruli, representing 60% of the total, associated with a degree of renal atrophy considerably greater than in the three-week hydronephrotic kidney, would be expected to show in over-all titer a greater decrease in concentration of antigen(s). There is at least

one factor that might be concerned with the lack of this expectation. Renal ischemia of the order induced here is undoubtedly associated with a fall in intrarenal pressure. There is divergence of opinion as to the extent of change in intrarenal pressure with varied degrees of venous obstruction and arterial ischemia as recently expressed by Swann, Moore, and Montgomery [34] and by Gottschalk.[35] Nevertheless, when arterial ischemia is such as to produce necrosis and profound atrophy, both groups of investigators would agree that there is an appreciable reduction in intrarenal pressure. This reduction would in part counteract the diminished tension within the basement membrane occasioned by the lowered intraglomerular capillary pressure of the surviving glomeruli. It is possible, too, that the expected reduction in volume and rate of blood flow might be of such degree as to reduce the metabolic activity of the membrane to the point where there may be little change in antigenic concentration.

While little is known about the role of the "nephrotoxic" antigen(s) of the glomerular basement membrane in the development of spontaneous glomerulonephritis, it is nevertheless of interest to draw attention to the following possible correlations: It has been shown here that venous congestion is associated with increased concentration of the "nephrotoxic" antigen(s). It is possible that the elevated venous pressure so commonly accompanying subacute bacterial endocarditis may in some way, as in the manner described above, render the glomeruli more susceptible to the development of the frequently associated glomerulonephritis in this disease. The same principle may influence the occurrence of glomerulonephritis in conjunction with large femoral arteriovenous fistulae produced in dogs by Lillehei and associates.[36]

On the other hand, the reduction in "nephrotoxic" antigen(s) associated with compensatory renal hypertrophy may serve as a protective mechanism against the development of glomerulonephritis. The conjunction of the infrequency in humans of unilateral hypertrophied kidneys and the relative infrequency of spontaneous glomerulonephritis makes it unlikely that a body of statistical evidence could be collected to demonstrate the above point. There are, however, only two case reports in the literature that we have been able to find in which diffuse glomerulonephritis occurred in a unilateral hypertrophied kidney only. In Fahr's [37] case there was atrophic hydronephrosis of the contralateral kidney, and in Paul's,[38] an old thrombotic occlusion of the renal artery with atrophy of the contralateral kidney. The advantage of a decreased antigenic concentration with compensatory hypertrophy could, however, be offset by more antibodies being brought to the glomerular sites by the larger volume of blood flowing through these kidneys.

CONCLUSIONS

The concentration of "nephrotoxic" antigen(s) in the glomerular basement membrane of the developing kidney in the dog increases commensurate with the maturation of the glomeruli. The concentration is presumably very low in the formative glomeruli of the nephrogenic zone. It rises abruptly when the glomeruli become capable of functioning as filters in the midzone of the neonatal cortex and has apparently not quite reached adult levels in the larger glomeruli of the juxtamedullary zone.

The concentration of "nephrotoxic" antigen(s) in adult canine glomerular basement membrane is apparently increased more than threefold to fivefold by venous congestion and in the presence of marked hydronephrosis. It is decreased twofold to threefold, or more, in association with compensatory renal hypertrophy. There is only slight reduction in concentration with marked ischemia, induced by clamping the renal artery.

These variations in concentration of the "nephrotoxic" antigen(s) are thought to be the result of physical and chemical changes within the glomerular basement membrane, stemming from altered tension created within it by changes in pressure and flow relationships. For these variations to occur there must be a fairly adequate blood supply, since profound ischemia apparently inhibits metabolic changes within the basement membrane, fixing the amount of antigen at its original level.

Two additional findings are of interest:

(*a*) There is a much shorter latent period in the development of "nephrotoxic" serum glomerulonephritis in the newborn than in the adult dog.

(*b*) There is increased fragility of the glomerular basement membrane accompanying immaturity and atrophy of the glomeruli.

Mr. A. Goode gave technical assistance.

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EFFECTS OF GLUCOCORTICOIDS AND MINERALOCORTICOIDS ON DEHYDROGENASE ACTIVITY OF ADRENAL SLICES

In Vitro Experiments with Control and Tumor-Bearing Mice

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THE ABILITY of surviving tissue slices to reduce 2, 3, 5-triphenyltetrazolium chloride (TTC) to a red water-insoluble formazin has been employed to measure in vitro dehydrogenase activity quantitatively and to visualize such activity histochemically.[1] Such techniques have been applied to the problem of adrenocortical metabolism in health and disease.* More recently we have reported a study of the in vitro endogenous dehydrogenase activity of adrenal slices from control and tumor-bearing mice.[4] As judged by the endogenous reduction of TTC, the adrenal slices from male and female control mice differed from each other and from females bearing spontaneous mammary carcinomas. The pertinent findings included a slighter degree of dehydrogenase activity in the zona glomerulosa and zona reticularis of adrenal slices from the female controls than from the male controls, whereas adrenal slices from female tumor-bearing mice possessed a well-defined dehydrogenase activity in the zona glomerulosa and zona reticularis, as well as some increase in such enzymatic activity in the zona fasciculata.

In order to explore this line of study further, it seemed pertinent to determine the direct effects of various steroid hormones on the in vitro dehydrogenase activity of adrenal slices in control and tumor-bearing mice. While the zona fasciculata is generally held to be concerned with glucocorticoid production under the direct trophic control of the anterior pituitary, there is evidence that the zona glomerulosa is more concerned with salt-water metabolism and possesses a greater degree of autonomy.[5] It therefore seemed advisable to initiate the study by determining the effects of cortisone (Compound E. of Kendall) and Compound F (17-hydroxycorticosterone), potent glucocorticoids, as well as desoxycorticosterone acetate (DOCA), a synthetic mineralocorticoid, on the in vitro dehydrogenase activity of adrenal slices.

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The investigation was supported by the Helen Andreadis Foundation, Inc., and the Leukemia Research Foundation, Inc.

* References 2 and 3.

Our findings provide further evidence of differences in the metabolic activity of the adrenals of male and female control mice, as well as indication of functional changes in the metabolism of the adrenals of female mice bearing spontaneous mammary tumors.

MATERIAL AND METHODS

In the present study three series of CFW mice were employed. They consisted of 29 control nongravid females weighing 20.8 to 32.4 gm. (mean weight, 25.8 gm.), 19 control males weighing 23.6 to 41.7 gm. (mean weight, 30.4 gm.), and 20 non-gravid females bearing spontaneous mammary tumors. The tumor mice weighed between 26.3 and 45.4 gm. (mean weight, 34.9 gm.), while the tumor weights varied from 0.1 to 4.4 gm. (mean weight, 1.4 gm.) excepting one tumor, which weighed 12.0 gm.

The animals were killed by rapidly crushing the cervical spine. The adrenals were then removed, gently cleared of their surrounding fat, and bisected with a razor, and one-half of an adrenal was placed in the appropriate incubation solution.

The incubation solution employed for the determination of the in vtiro endogenous dehydrogenase activity consisted of 3 ml. of a 1% aqueous solution of TTC buffered to pH 7.2 and 1 ml. of 0.9% sodium chloride. In the studies with the steroid hormones, 1 ml. of a supersaturated aqueous solution of crystalline hormone† was substituted for the isotonic saline. The final mixture thus obtained yielded a fine dispersion containing approximately 0.45 mg. of cortisone acetate, 0.21 mg. of desoxycorticosterone acetate, and 0.1 mg. of Compound F in the respective incubation media. Measurement of the pH of the various incubation media yielded values of 7.2, with the exception of the solution containing Compound F, of which the pH was 7.1.

After incubation for one hour at 37 C., the reactions were stopped by fixing the tissues in 10% formalin. The tissues were allowed to fix overnight, during which time they were stored in the dark. The prolonged fixation was employed to facilitate the preparation of frozen sections and did not alter the appearance of the formazin deposition. After fixation, frozen sections were prepared. In almost all cases four sections from each adrenal slice were examined. A direct visual comparison was then made of the intensity of the dehydrogenase activity in the various zones of the same section and between the endogenous activity and that observed in the presence of the steroid hormones studied. In addition, color photomicrographs were taken of each case, so that a permanent record was available for further study.

It is pertinent to state that in our previously reported studies of mouse adrenal slices we had compared the appearance of the gland after routine hematoxylin and eosin staining and Sudan staining for lipid with that observed after TTC incubation. It appeared that while the TTC reduction in such slices was closely associated with the lipid granules of the cells, the TTC technique was a more sensitive index of adrenocortical function under diverse physiological and pathological situations.‡

RESULTS

Male Controls.—As described in our previous report,[4] the in vitro endogenous dehydrogenase activity of adrenal slices from male control mice, as visualized by TTC, is characterized by a well-defined red staining of the lipid granules in all zones, the zona fasciculata being somewhat more prominent than the others (Fig. 1).

In those slices incubated with TTC plus cortisone, there was no appreciable difference in the pattern of formazin deposition. However, an increased intensity of staining was noted in the zona fasciculta and, to some extent, in the zona glomerulosa (Fig. 2). An increased intensity of staining was also observed in the adrenal slices incubated with TTC plus Compound F. In fact, the staining in the latter group appeared to be somewhat more pronounced than that found in the cortisone studies.

† The crystalline cortisone and DOCA were supplied by Charles Pfizer & Company, Inc., through the courtesy of Dr. I. V. Sollins.

‡ References 2 through 4.

On the other hand, when slices were incubated in the presence of DOCA, there was a definite inhibition of the zona glomerulosa and usually the outer part of the zona fasciculata as well (Fig. 3).

Fig. 1.—Adrenal, male CFW mouse. Endogenous dehydrogenase activity. Active reduction of TTC by cells of zona glomerulosa and zona fasciculata. × 440.

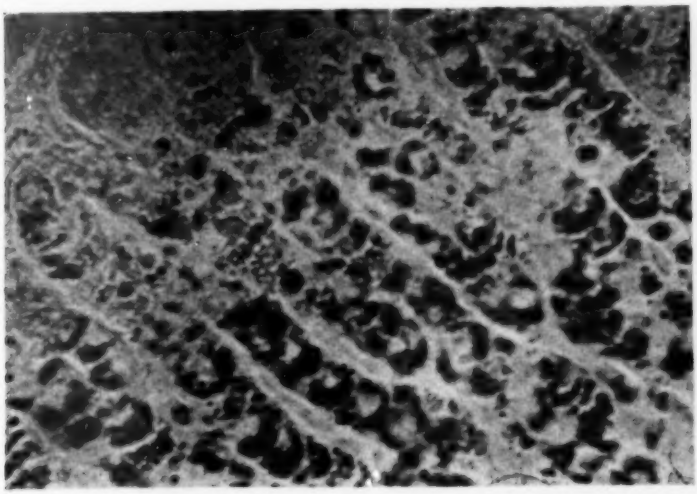

Fig. 2.—Adrenal, male CFW mouse. Dehydrogenase activity in presence of cortisone. Increased intensity TTC reduction by zona glomerulosa and zona fasciculata, as evidenced by deeper red coloration of intracytoplasmic lipid granules. × 440.

Female Controls.—The in vitro endogenous dehydrogenase activity of adrenal slices from female CFW control mice is predominantly confined to the cells of the zona fasciculata, minimal to no TTC reduction being observed in the zona glomeru-

losa and zona reticularis (Fig. 4). It should be noted, however, that in breeder females older than 10 months of age the zona reticularis was found to contain lipid granules which stained with TTC. In addition, in many instances the innermost

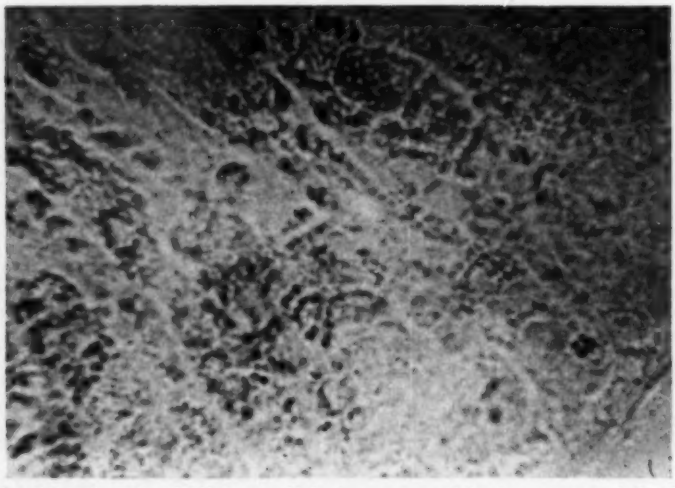

Fig. 3.—Adrenal, male CFW mouse. Dehydrogenase activity in presence of DOCA. Suppression of TTC reduction by cells of zona glomerulosa and outermost zona fasciculata. Note difference from effect in cortisone study. × 440.

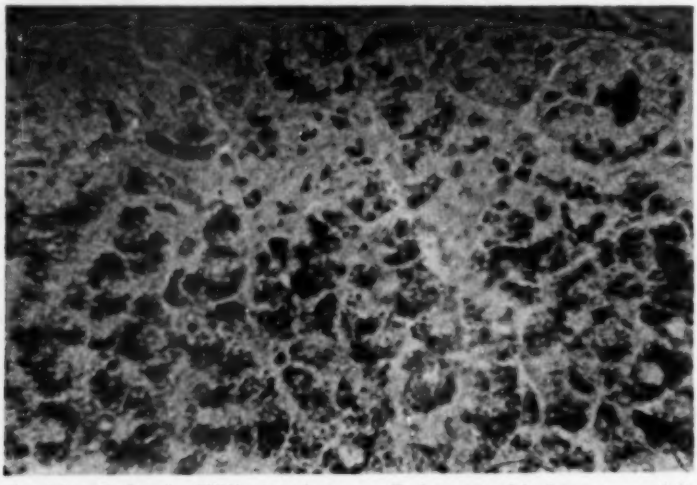

Fig. 4.—Adrenal, female CFW control mouse. Endogenous dehydrogenase activity. TTC reduction by cells of zona glomerulosa somewhat more marked than in average case. × 440.

cells of the zona reticularis exhibited a granular nonlipid intracytoplasmic formazin deposition, which appeared to be accentuated by cortisone and Compound F.

In those slices incubated in the presence of cortisone, the zona fasciculata showed a slight increment in the intensity of the staining as compared with the endogenous

studies. The zona glomerulosa also appeared to be stimulated, so that a well-defined staining became evident in some of the cases. On the other hand, no change in staining, aside from that mentioned above, was observed in the zona reticularis (Fig.

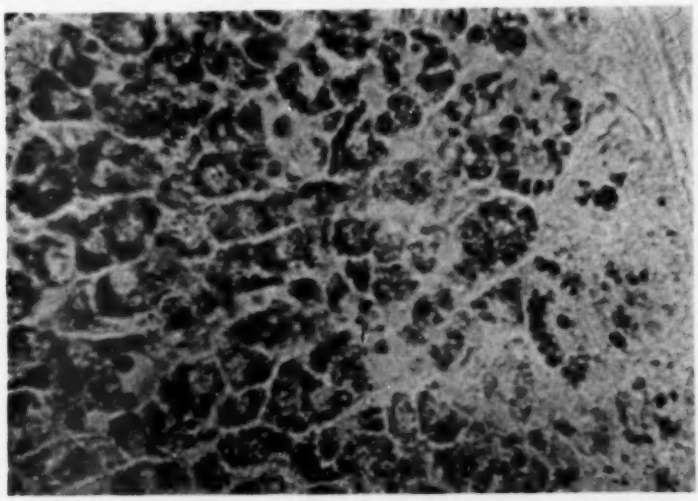

Fig. 5.—Adrenal, female CFW control mouse. Dehydrogenase activity in presence of cortisone. Zona glomerulosa shows definite TTC reduction. × 440.

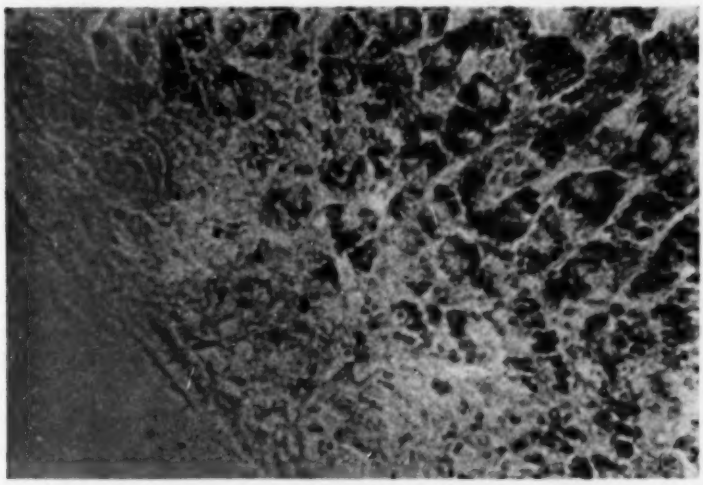

Fig. 6.—Adrenal, female CFW control mouse. Dehydrogenase activity in presence of DOCA. Note lack of TTC reduction in zona glomerulosa and decreased activity in outermost zona fasciculata. Note difference from activity in cortisone study. × 440.

5). Similar findings were obtained in the studies with Compound F. As with the male mice, the Compound F seemed to produce a somewhat greater increment in staining than was observed with cortisone.

After incubation with DOCA, the adrenal slices from the female mice usually showed no formazin deposition in the zona glomerulosa. The DOCA inhibition of the zona fasciculata in the female group was not so pronounced as that observed in the male group. However, when observed, the DOCA effect on the zona fasciculata was most marked in the outermost portion of this zone (Fig. 6).

Females with Spontaneous Mammary Tumors.—As previously reported, all cortical zones of the adrenal slices from mice with spontaneous mammary tumors showed an active in vitro endogenous reduction of TTC (Fig. 7).

After incubation with cortisone, the TTC reduction by the zona glomerulosa was definitely less active, and to a less extent, so was that of the zona fasciculata, while the zona reticularis remained unchanged (Fig. 8). A similar, and even more pronounced, inhibition was observed in the slices exposed to Compound F.

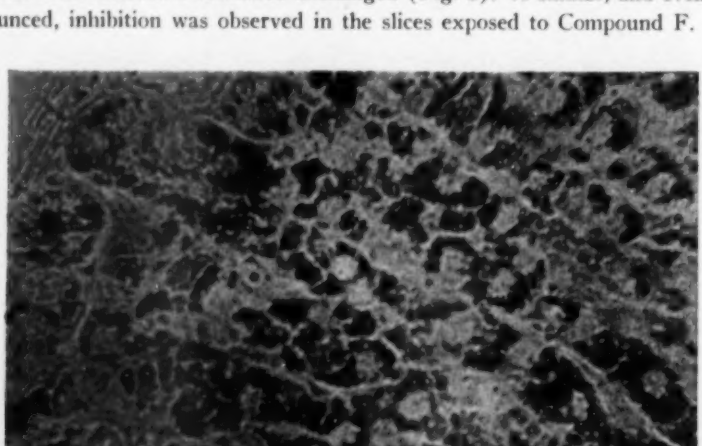

Fig. 7.—Adrenal, female CFW mouse with spontaneous breast tumor. Endogenous dehydrogenase activity. Active TTC reduction by zona glomerulosa and zona fasciculata. × 440.

In contrast, DOCA did not cause any appreciable inhibition and in some cases even seemed to stimulate the zona glomerulosa and the outermost zona fasciculata (Fig. 9). Thus, the essential difference between the control and the tumor-bearing mice in regard to their response to the hormones was the finding that in the control group the staining of the zona glomerulosa and the outer portion of the zona fasciculata was much less intense in the DOCA preparations than in the cortisone-exposed or Compound F-exposed slices, whereas in the tumor group the reverse was the rule.

In order to obtain some degree of quantitation of the aforementioned histochemical findings, the intensity of the staining of the various cortical zones was graded from 0 to 4+. A bright red color was assigned a reading of 4+; the absence of color was graded as 0, while intermediate intensities of color were assigned values

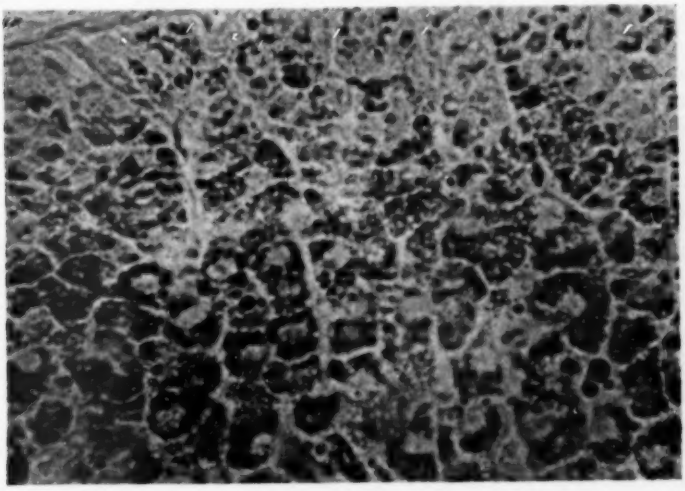

Fig. 8.—Adrenal, female CFW mouse with spontaneous breast tumor. Dehydrogenase activity in presence of cortisone. Note decreased TTC reduction in zona glomerulosa and outermost zona fasciculata. × 440.

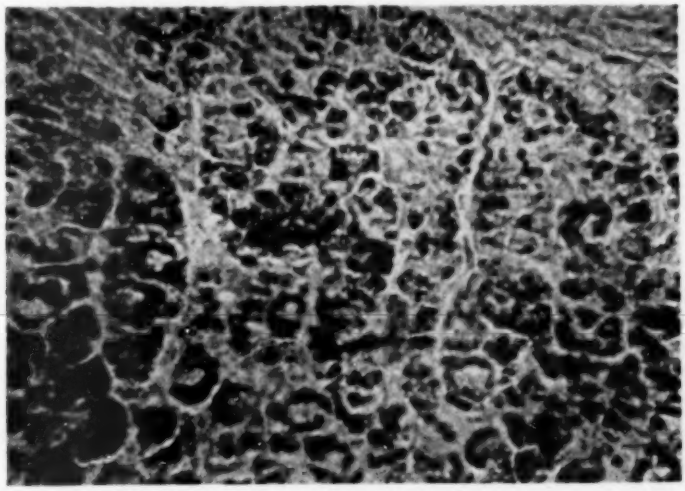

Fig. 9.—Adrenal, female CFW mouse with spontaneous breast tumor. Dehydrogenase activity in presence of DOCA. Intensity of TTC reduction by zona glomerulosa and zona fasciculata, similar to that in the endogenous preparation. Contrast DOCA effect in tumor group with that observed in controls. × 440.

in accord with their relation to the two extremes of the scale. In Figure 10 we have presented diagrammatic profiles based on the mean values obtained in accord with the above system of grading. These data provide a semiquantitative comparison of the effect of DOCA on the in vitro dehydrogenase activity of the adrenal slices from the tumor-bearing mice and the effect on the controls. It is evident that the differential action is found principally in the zona glomerulosa, less in the zona fasciculata, and minimally in the zona reticularis. The difference between the effects of cortisone and Compound F on the adrenals from the tumor mice and the effects on the controls is also readily apparent. Here, too, the zone exhibiting the maximal difference is the zona glomerulosa.

A comparison of the intensity of the dehydrogenase activity of the zona glomerulosa in the presence of the glucocorticoids and DOCA is of interest. As depicted in Figure 10, in the male control group the mean values for the dehydrogenase activity of the zona glomerulosa in the cortisone and Compound F studies were both more than eight times that observed after DOCA incubation. In the female control group

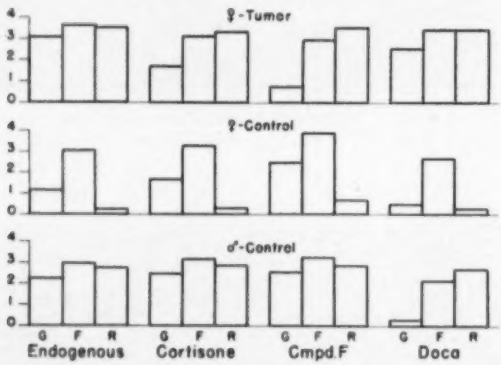

Fig. 10—Diagrammatic representation of the relative intensity of the in vitro dehydrogenase activity of various zones of adrenocortical slices from control and tumor CFW mice in the presence and absence of cortisone, Compound F, and DOCA. *G* indicates zona glomerulosa; *F*, zona fasciculata, and *R*, zona reticularis.

the activity of the zona glomerulosa after exposure to cortisone and Compound F was three and five times as great respectively as that in the DOCA incubation studies. In contrast, similar comparisons in the tumor group revealed that exposure to cortisone and Compound F yielded mean values which were less than that obtained after DOCA incubation, i. e., cortisone 1.8, Compound F 0.8, and DOCA 2.6 units.

It is also noteworthy that this change in the relative effect of the hormones on the metabolic activity of the zona glomerulosa was not dependent upon the presence of metastatic involvement; if anything, the reverse appeared more likely. In the present series pulmonary metastases were noted in four of the mice. In this group the mean values for the intensity of the dehydrogenase activity of the zona glomerulosa under the conditions of study was found to be as follows; endogenous, 2.8; cortisone, 1.8, and DOCA, 2.0 units. In the remaining 16 cases the corresponding values were 3.2, 1.9, and 2.7 units.

These findings leave little doubt that the presence of spontaneous mammary tumors in female CFW mice is associated with changes in the metabolic activity of

the adrenal cortex which appear to have an appreciable degree of selectivity in regard to the cortical zones.

Medulla.—The medullary cells failed to exhibit detectable in vitro endogenous dehydrogenase activity. However, in some instances a definite fine granular staining was found in the cytoplasm of the medullary cells from the female control mice after incubation with Compound F. Since such findings were not constant, it would be premature to attempt any finite interpretation at this time.

COMMENT

In a previous study we had observed that the implantation of DOCA pellets into rats led to an inhibition of the in vitro dehydrogenase activity of the zona glomerulosa without affecting such enzymatic activity in the other cortical zones.[2] On the other hand, cortisone injections caused no appreciable effect on the TTC reduction by the zona glomerulosa, although the zona fasciculata and zona reticularis underwent a progressive loss in their in vitro ability to reduce TTC, resulting in an appearance similar to that observed after hypophysectomy. In addition, we found that implantation of sarcoma 180 into female and male mice led to a similar decrease in the in vitro dehydrogenase activity of the adrenal cortex, which proceeded from the inner to the outer zones and was followed by a progressive loss of intracellular lipid.[4] However, this process did not involve the zona glomerulosa and, in fact, was associated with an increase in the dehydrogenase activity of this zone in the adrenals of female mice with the implanted tumors. Such observations are indicative of a relative independence of function of the zona glomerulosa as compared with the other cortical zones. The results of the present investigation provide further evidence that the controlling factors involved in the metabolic activity of the zona glomerulosa differ from those of the zona fasciculata. Thus, the dehydrogenase activity of the zona glomerulosa of the control mice was directly inhibited by DOCA. In contrast cortisone and Compound F, which are capable of inducing a profound inhibition of the zona fasciculata when injected into intact animals, failed to inhibit the dehydrogenase activity of this zone in the incubation experiments. In fact, the glucocorticoids acted more like substrates, since they tended to increase the intensity of the TTC reduction. These findings are in accord with the generally held concept that the cortisone or Compound F inhibition of the adrenocortical function in intact animals is mediated through their depression of pituitary corticotropin production. On the other hand, it appears that the zona glomerulosa is more directly responsive to the level of its circulating hormone and/or the salt concentration.

As reported previously and confirmed in the present study, the in vitro endogenous dehydrogenase activity of the adrenals of tumor-bearing CFW female mice was readily distinguished from that of control females by virtue of the prominent staining of the zona glomerulosa and zona reticularis in the former. Such findings, if considered alone, might be taken to indicate a simple quantitative increase in the activity of the zona glomerulosa and zona reticularis in the presence of a tumor. However, when we consider that DOCA failed to inhibit the zona glomerulosa, and in many cases even stimulated the dehydrogenase activity of this zone, it becomes evident that a qualitative, as well as a quantitative, change in metabolism must be envisioned. The lack of inhibition by DOCA might possibly be due to either (*a*) the loss of the

autonomous function of the zona glomerulosa or (*b*) a change in the synthetic activity of the zona glomerulosa, so that it no longer produced salt-water metabolic hormones, and would, thus, no longer be inhibited by such hormones. However, the finding that the glucocorticoids, cortisone, and Compound F tended to inhibit the zona glomerulosa selectively is more in keeping with the latter alternative. In short, the present findings would be consistent with the hypothesis that the adrenals of the tumor-bearing mice had undergone a change in the metabolism of the zona glomerulosa associated with which there was change in its hormone synthesis in the direction of producing less salt-water metabolic steroids and more of the catabolic glucocorticoids. It should be noted that such an explanation would be consistent with the clinical observations that indicate a salt-water metabolic defect and a tendency to hypotension in cancer patients.§

Finally, it should be pointed out that these changes in the adrenocortical metabolism of the tumor-bearing mice were not associated with cachexia of the host. In most instances the tumors represented less than 5% of the body weight and the mice appeared to be in good health. These subtle, but complex, changes in adrenocortical metabolism associated with the presence of spontaneous tumors appear to be further indications of a systemic component in malignant disease.

SUMMARY

A histochemical study was made of the in vitro dehydrogenase activity of adrenal slices of CFW mice utilizing the tetrazolium salt 2, 3, 5-triphenyltetrazolium chloride (TTC). A comparison was made between the intensities of the metabolic activity of the various cortical zones as found in adrenals from male and female controls and females bearing spontaneous mammary cancer. In addition, an evaluation was made of the relative effects of cortisone, Compound F, and desoxycorticosterone acetate (DOCA) on the dehydrogenase activity in the above groups.

The dehydrogenase activity of the zona glomerulosa of adrenal slices from the control animals was characterized by a tendency to be inhibited by DOCA and stimulated by the glucocorticoids. In contrast, the dehydrogenase activity of the zona glomerulosa of adrenal slices from the tumor mice tended to be inhibited by the glucocorticoids and unaffected or stimulated by DOCA. To some extent the findings in the outermost zona fasciculata tended to parallel those observed in the zona glomerulosa. No appreciable changes were noted in the inner zona fasciculata or zona reticularis. These findings are discussed in regard to their possible implications.

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LEIOMYOMA OF THE LUNG

Report of a Case

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LIEUTENANT ROBERT L. ALZNAUER (MC), U.S.N.R.
AND
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PRIMARY leiomyoma of the lung is a rare tumor. Five well-documented cases have been reported in the literature (Table). The first was reported by Forkel in 1909,[1] the second by Franco in 1929,[2] the third by Brahdy in 1941,[3] the fourth by Williams and Daniel in 1950,[4] and the fifth by Freireich, Bloomberg, and Langs in 1951.[5] Although a case of "multiple primary myomas of the lung" in a 57-year-old woman reported by Duessing in 1912 [6] has been accepted both by Brahdy and by Williams and Daniel, there is considerable doubt as to its validity, since multiple myomas of the uterus were also present. In view of the multiplicity of the pulmonary lesions, the possibility that these might be pulmonary metastases from a well-differentiated leiomyosarcoma of the uterus cannot be excluded. The validity of Duessing's case was also questioned for the same reason by Franco in 1929,[2] and by Randall and Blades in 1946.[7] In a series of 67 "solitary intrapulmonary lesions" reported by Davis and Klepser in 1950,[8] five of them were said to be "myofibromas." Since this paper was written with the primary emphasis on peripheral bronchiogenic carcinoma, complete descriptions and photomicrographs of these cases were not presented, and for this reason they are not included in this report. For the same reasons the four cases of "myoma" and three cases of "myosarcoma" reported by Sherman and Malone in 1930 [9] are not included. To the best of our knowledge this is the sixth case of primary benign leiomyoma of the lung to be reported.

REPORT OF A CASE

History.—E. B., a 24-year-old white man, was admitted to the United States Naval Hospital, San Diego, Calif., on Aug. 21, 1951, because an "isolated pulmonary nodule" was discovered in the right lung field in a routine chest roentgenogram taken at the time of his discharge from the Naval Service. He had been completely asymptomatic. Physical examination revealed no

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The opinions presented are those of the authors and do not reflect the views of the Medical Department of the United States Navy.

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abnormal findings. The usual admission laboratory tests, which included a blood count and urinalysis, were within normal limits, and the serologic test for syphilis was negative. Bronchoscopic examination revealed no abnormalities, and smears of the bronchial washings stained by the Papanicolaou technique showed no abnormal cells. Smears and cultures for acid-fast bacilli of one bronchial wash and three gastric washes were negative. Routine skin tests, purified protein derivitive of tuberculin in first and second strengths, coccidioidin 1:100, and histoplasmin 1:100, were all negative. Gastrointestinal tract examination by fluoroscopy and x-ray, cystoscopy, and intravenous and retrograde pyelograms revealed no abnormalities. The Ascheim-Zondek test for urinary gonadotropins showed less than 33 M.U. per 24 hour specimen. Chest x-rays (Figs. 1 and 2) taken on Aug. 22, 1951, revealed a "soft round cotton-ball" in the right middle lobe, which measured 3 cm. in diameter. The problem in roentgen diagnosis was "metastasis versus inflammatory granuloma." Since the etiology of the pulmonary lesion remained undetermined, exploratory thoracotomy was carried out on Sept. 27, 1951, and a right middle lobectomy was performed. The postoperative course was uneventful, and the patient was discharged as a civilian on Nov. 28, 1951.

Gross Pathological Examination.—The specimen consisted of the middle lobe of the right lung, which measured 10 by 7 by 3 cm. and weighed 80 gm. The pleura was smooth and

Summary of Reported Cases

Author Date	Sex	Age, Yr.	Anatomical Location Size of Tumor	Symptoms	Operation	Remarks
Forkel 1909	F	63	Parenchymal; left upper lobe "lemon" size	None	Incidental at autopsy	Diagnosis verified by differential stains
Franco 1929	F	56	Parenchymal; right upper lobe 13 × 9 cm.	?	Autopsy finding	Diagnosis verified by differential stains
Brahdy 1941	F	18	Parenchymal; right lower lobe 2.5 cm.	None per se; patient had tuberculosis	Right lower lobectomy	Diagnosis verified by differential stains
Williams & Daniel 1950	F	8	Parenchymal; left lower lobe 10 cm.	Chronic cough productive of mucoid sputum	Left pneumonectomy	Diagnosis verified by differential stains
Freireich, Bloomberg, & Langs 1951	M	61	Polypoid intrabronchial lesion; right upper lobe 1 cm.	None	Right upper lobectomy	Diagnosis verified by differential stains
Pieree, Aimauer, & Rolle 1953	M	24	Parenchymal; right middle lobe 3 cm.	None	Right middle lobectomy	Diagnosis verified by differential stains

glistening. The parenchyma was collapsed and noncrepitant, with a dusky-red cut surface. An oval tumor nodule was present in the parenchyma in the central portion of the lobe. It measured 3 by 2 by 2 cm. and was sharply demarcated from the adjacent parenchyma. The bulging cut surface was firm and gray-white and had a whorled appearance. No relationship to adjacent bronchi could be established grossly.

Histology.—Sections of lung (Figs. 3 and 4) revealed a well-differentiated neoplasm which was composed of interlacing whorled bundles of elongated spindle-shaped cells. In some areas these were also arranged in a "herring-bone" pattern. The narrow elongated nuclei were pale and vesicular and showed no pleomorphism or mitotic activity. The pink cytoplasm took up a characteristic brick-red color with Masson's trichrome stain. Hence, the tumor cells were interpreted as myofibrils of smooth muscle type. Near the periphery of the tumor in some areas there was an infiltrate of small mononuclear cells which were predominantly plasma cells. No relationship with the wall of a bronchus or blood vessel could be established. The adjacent pulmonary parenchyma showed no significant histological change.

Diagnosis.—The diagnosis was benign leiomyoma of the middle lobe of the right lung.

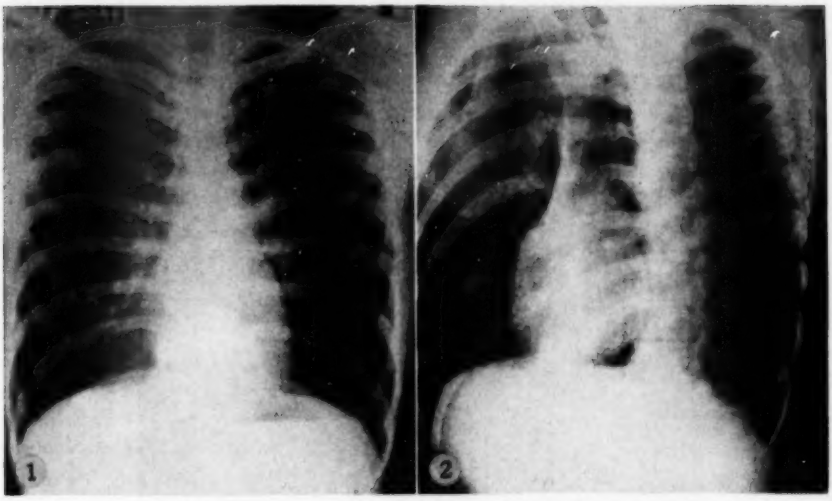

Fig. 1.—X-ray of the chest, posteroanterior view, showing the discrete solitary nodule in the lower portion of the right lung.

Fig. 2.—X-ray of the chest, left anterior oblique view, demonstrating the anterior location of the solitary nodule in the middle lobe of the right lung.

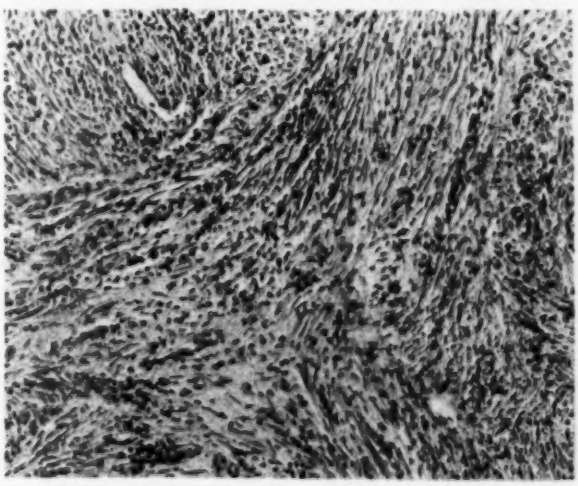

Fig. 3.—Leiomyoma of the lung. Low-power view showing the interlacing whorled arrangement of the spindle-shaped myofibrils. × 140.

COMMENT

The leiomyoma reported by Freireich, Bloomberg, and Langs [5] was intrabronchial in location. The other five were located in the pulmonary parenchyma. Two cases were asymptomatic and were detected by routine chest x-ray. In view of mass chest x-ray surveys now being conducted, additional cases will undoubtedly be discovered because of the increasing trend toward removal of indeterminate lesions of the lung. Surgical removal of these tumors, even if they are asymptomatic, is sound treatment. Nine cases of primary leiomyosarcoma of the lung have been reported, one each by Brunn and Goldman in 1940,[10] Randall and Blades in 1946,[7] and Johnson, Mangiardi, and Jacobs in 1952,[11] and six by Watson and Anlyan in 1954.[12] Since leiomyosarcomas can arise in preexisting leiomyomas of the uterus, it is reasonable that malignant change could occur in a preexisting benign leiomyoma of the lung.

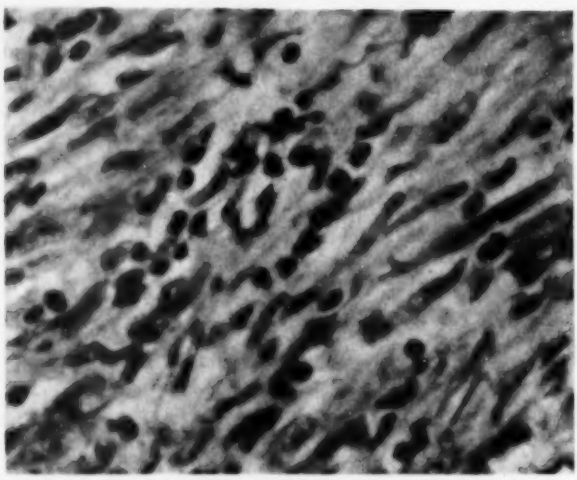

Fig. 4.—High-power view showing the detailed histologic appearance of the tumor cells. Note the uniform character of the elongated narrow vesicular nuclei. × 600.

One potential pitfall in establishing the correct diagnosis of this pulmonary neoplasm must be kept in mind. Willis [13] has emphasized that well-differentiated metastatic lesions in the lung, which have a histologically "benign" appearance, are known to occur from primary leiomyosarcoma of the uterus. These have been referred to as "benign metastasizing leiomyomas" by Steiner.[14] If a solitary pulmonary metastasis only should occur in such a case, it would be difficult to render a diagnosis of malignancy on the histologic pattern alone. In the case of women, one must especially rule out the presence of uterine leiomyomata as a possible source of a metastatic pulmonary lesion. In all cases, however, leiomyosarcoma of the gastrointestinal tract, retroperitoneal tissues, etc., must be considered and excluded.[15]

In the histological study of this tumor it is essential that special staining techniques, either Masson's trichrome or Van Gieson's acid fuchsin methods, be carried out to establish the exact nature of the spindle cells. The neoplasm in our case was

originally considered to be a neurofibroma until the trichrome stain demonstrated a brick-red cytoplasm in the tumor cells. Primary neurogenic neoplasms are rare, but they do occur in the lung parenchyma. Three such cases, which were interpreted as neurofibromas, have been reported by Bartlett and Adams in 1946,[16] Touroff and Sapin in 1949,[17] and Diveley and Daniel in 1951.[18] In addition, Lane, Murray, and Fraser reported a case of neurilemoma of the lung in 1953.[19] They were also able to review the slides of the other three cases through the courtesy of the above authors and concurred in the diagnosis of neurofibroma in each case. They did not, however, consider them to be neurilemomas like their own case. Since both smooth muscle and neurogenic tumors frequently show similar features, such as palisading of the nuclei and whorling of the spindle cells, the only certain means of differentiation is by special staining techniques. This similarity between leiomyomas and neurogenic tumors due to palisading of nuclei is also emphasized by Lane, Murray, and Fraser,[19] who state that this may be a source of error in diagnosis.

SUMMARY

A case of primary benign leiomyoma of the lung is presented. The patient was asymptomatic, and the lesion was discovered in a routine roentgenogram of the chest.

Review of the literature reveals five similar cases which are well-documented, as well as nine cases of primary leiomyosarcoma of the lung.

The possibility of an extrapulmonary leiomyosarcoma with a histologically "benign"-appearing solitary metastasis to the lung must always be considered and ruled out by thorough examination of all other organ systems.

The necessity for thorough histological study of these mesenchymal pulmonary neoplasms by means of special staining techniques is emphasized.

Donald E. Reeves, HM$_2$, U. S. N., of the Pathology Service, United States Naval Hospital, San Diego, Calif., prepared the Masson trichrome stains, and William Von Allmen, HM$_3$, U. S. N. R., of the Medical Photography Service, reproduced the photographs of the x-ray films.

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EFFECT OF CORTISONE ON CARBON TETRACHLORIDE CIRRHOSIS IN RATS

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INHIBITION of the progressive fibroplasia of hepatic cirrhosis was suggested by the observations that cortisone and corticotropin (ACTH) depress the formation of scar tissue.* Consequently, this investigation was initiated to study the effect of cortisone on carbon tetrachloride cirrhosis in rats. While these experiments were being carried out, several articles were published reporting the suppressing effect of cortisone on hepatic fibrosis induced by carbon tetrachloride in rats.† The results of the present investigation, however, have shown that cortisone exerts a deleterious influence on the liver during carbon tetrachloride poisoning and enhances the fibrosis.

MATERIALS AND METHODS

Twenty male albino rats, initially weighing 150 to 190 gm., maintained on a regular diet, were prepared by exposure to carbon tetrachloride vapors three times weekly, according to the method of Morrione.[6] After the 10th inhalation period (i. e., on the 21st day of the experiment), 13 rats received cortisone acetate and at the same time exposure to carbon tetrachloride was continued. Cortisone (Cortone) was given by subcutaneous injection twice daily, according to the following schedule: 5 mg. daily for six consecutive days, 10 mg. daily for the following three days, and 5 mg. daily for the last two days of the experiment. Five rats died during the early stages of cortisone treatment and were discarded. The remaining surviving animals were examined after having received a total of 67.5 to 70 mg. of cortisone and 15 inhalations of carbon tetrachloride. Control rats, exposed to the same number of carbon tetrachloride inhalations, received injections of isotonic saline solution twice daily.

Rats treated with cortisone showed weight losses of 30 to 60 gm.

For histological study two blocks from different lobes of the liver were fixed in Zenker's acetic acid. Paraffin sections were stained with hematoxylin and eosin and with Laidlaw's silver method, Van Gieson's stain being used for counterstaining.

FINDINGS

1. *Controls.*—The livers of all seven control rats showed similar and uniform changes (Fig. 1). The normal architecture were replaced by large pseudolobules which were only partially surrounded by thin fibrosis. The fibrous septa were

This investigation was supported in part by the Hadassah Medical Organization Clinical Research Fund.

From the Department of Medicine and the Department of Pathology, Hadassah University Hospital and the Hebrew University, Hadassah Medical School.

* References 1-3.

† References 4 and 5.

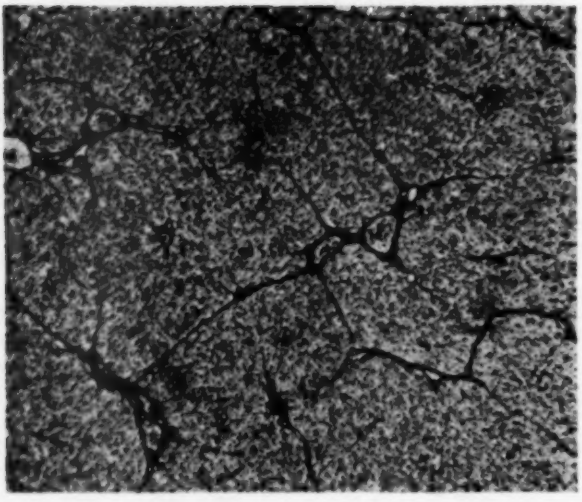

Fig. 1.—Characteristic field showing degree of cirrhosis at the end of the experiment in control rats. Laidlaw and Van Gieson stain; × 48.

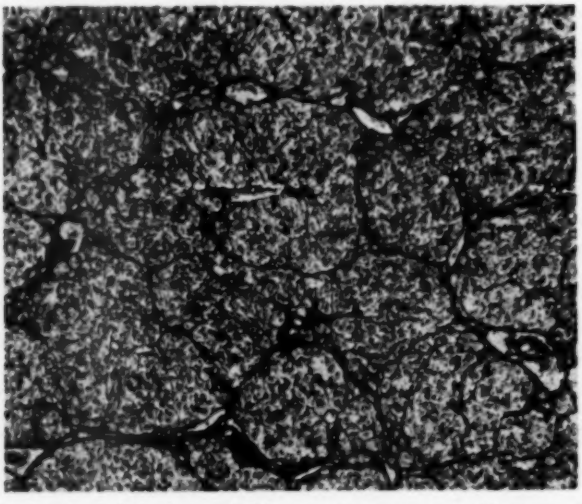

Fig. 2.—The degree of cirrhosis revealed in rats after treatment with cortisone. Laidlaw and Van Gieson stain; × 48.

moderately thick around the portal spaces and quickly tapered off within the parenchyma. They were composed of a small number of delicate straight reticulum fibers. Fibroblasts were numerous within the septa, and a cellular exudate was absent. The liver cells themselves showed no morphological changes.

2. *Cortisone Administration.*—In the cortisone-treated animals the liver showed a number of alterations distinctly different from those observed in the controls. The hepatic architecture was replaced by numerous pseudolobules which were one-third to one-fifth of the size of those in controls (Fig. 2). The surrounding fibrous septa were wider and contained numerous bile ducts and a moderate number of fibroblasts with hyperchromatic nuclei. Argyrophile fibers were found as densely packed bundles, which often enclosed groups of, and even individual, liver cells.

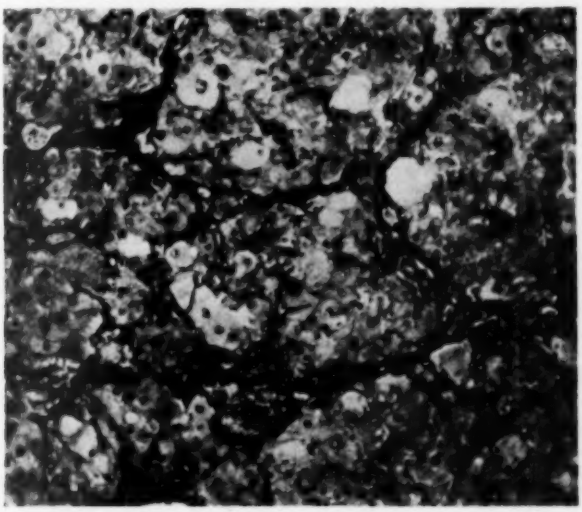

Fig. 3.—Coarseness and clumping of reticulum fibers after treatment with cortisone. Laidlaw and Van Gieson stain; × 150.

Clumping and coarseness of the reticulum fibers in the septal trabeculae was also conspicuous (Fig. 3).

The hepatic cells themselves varied markedly in size. Groups of multinucleated epithelial giant cells were scattered throughout the parenchyma. The number of nuclei ranged from 2 to 20 in a cell, occasionally clumped together or distributed around the periphery of the cytoplasm. When there were many nuclei in a cell they were considerably smaller than in the cells containing only a few. Within these giant cells, hyaline inclusions were occasionally found (Figs. 4-7).

In addition, there was diffuse moderate fatty changes within the parenchyma and foci of necroses.

COMMENT

The hepatic lesions were uniformly severer in all animals treated with cortisone than in the control rats, and this was evident in both the epithelial and the mesenchymal tissues.

The type of multinucleated giant cells which appeared after cortisone treatment have been described before in cases of severe hepatitis in infants,‡ and in rats given colchicine [11] or thiourea.[12] It has not been reported to develop after the use of carbon tetrachloride,§ or of cortisone alone, in doses employed in the present investigation.[5] In consideration of the above findings, this type of giant cell seemed to indicate an interference with regeneration of hepatic cells.

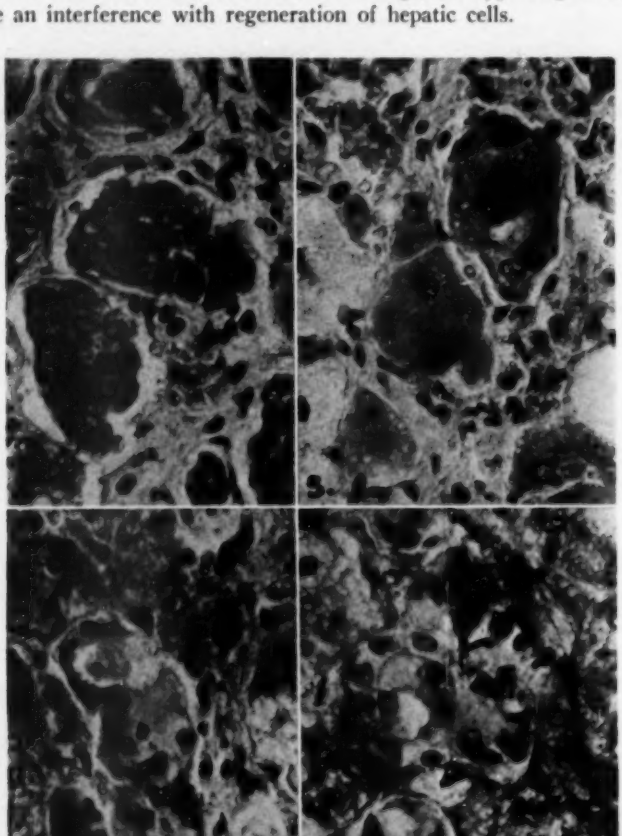

Fig. 4.—Various types of epithelial giant cells in the liver following treatment with cortisone. Figures 4-6 stained with hematoxylin and eosin; Figure 7 with Laidlaw and Van Gieson stain; × 560.

In cortisone-treated animals the connective tissue proliferated more abundantly than in controls, and it was composed of irregular hyperchromatic fibroblasts and dense bundles of coarse tortuous reticulum fibers. It was not determined whether

‡ References 7-10 and Anderson, D. H.: Discussion on Stokes.[10]

§ References 6, 13, and 14.

these mesenchymal alterations were the result of cortisone treatment or secondary to the severer parenchymal injury in the experimental animals. In any case, there was no inhibition of the cirrhotic process by cortisone, and, in fact, the hormone produced a severer change.

The present results were at variance with those of Aterman. Perhaps this is due to the preparation of the animals by repeated inhalations of carbon tetrachloride. This method, as suggested by Morrione,[6] produced regularly a uniform degree of hepatic fibrosis. On the other hand, prolonged administration of CCl_4 to rats by the subcutaneous route resulted in extremely varying degrees of cirrhosis.‖ The injection method was therefore of little value for therapeutic studies in rats.

SUMMARY

In albino rats exposed to vapors of CCl_4 the administration of cortisone enhanced the cirrhotic process in the liver considerably.

In comparison with the controls, the liver of cortisone-treated animals showed marked epithelial and mesenchymal alterations. The former was manifested by the presence of multinucleated giant cells, foci of necrosis, and fatty changes; the latter, by the development of dense bundles of tortuous and clumped reticulum fibers.

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CROSS CIRCULATION AND TISSUE REACTIONS IN PARABIOSIS

A Study of Cross Circulation and Tissue Reactions at Parabiotic Junctions in Rabbits

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HOMOGRAFTS of most normal tissues fail to survive in rabbits, for unknown reasons. It has been possible to distinguish between autologous and homologous musculofascial transplants in rabbits by the occurrence of lymphocytic infiltration, angiitis, decreased fibroblastic proliferation, and impaired collagen deposition in the subfascial zone of homologous transplants.[1] This interference with the normal process of healing was a consistent manifestation of the incompatibility between the tissues of one animal and those of another of the same species and strain. Also, the idea that this pattern of reaction might have an immunological basis was supported by demonstrating that the reaction developed as a more acute necrotizing response in homologous transplants in an animal which had previously received similar transplants from the donor.

In these experiments the development of the homologous reaction seemed to depend largely upon penetration of the musculofascial zone of the transplant by vascularized granulation tissue of the host. Inasmuch as this implied the excitation of an active unilateral response which primarily involved mechanisms of the host, it seemed desirable to study a bilateral response in which homologous reaction depended upon mutual interpenetration of vascularized granulation tissue at a common junction of tissues of rabbits in parabiosis. The present paper is concerned with the results of this study, which has disclosed some observations on the nature of the cross circulation at aural junctions of rabbit parabionts, the differences in healing at autologous and homologous aural junctions, and the systemic effects of parabiosis.

METHODS

Young male New Zealand rabbits weighing 2 to 3 kg. were used. Though these were from the same stock, they were not highly inbred. A lightweight plaster cast was placed around the neck and upper thorax of each animal four or five days prior to placement in parabiosis so as to allow each animal to become accustomed to its cast. Pairs of animals were then fastened together with adhesive tape applied to the casts.

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Surgical aural anastomosis was then done as follows.[2] The apposing ears were shaved and the skin prepared with aseptic technique. With the animals under procaine anesthesia, the distal 6 to 7 cm. of skin of the lateral aspect of each apposing ear was excised. A window was cut out of the exposed plate of cartilage, a peripheral rim of cartilage measuring about 0.5 cm. in width being left to support the ear and prevent curtailment of circulation following the anastomosis. The cartilages at the bases of the excised areas were united with interrupted monofilament sutures. The dissected areas were apposed and the anastomosis completed with interrupted monofilament sutures placed along the periphery of the ears. Therefore, the tissues placed in apposition consisted of skin, subcutaneous tissue, and cartilage of the two animals. After the anastomosis, the united ears remained erect and were free of foreign restraints. Freedom of movement of the heads and extremities of the parabionts was such that eating and drinking were unhindered.

Results of Use of Intravenous Dyes and India Ink to Demonstrate Presence of Cross Circulation Between Rabbit Parabionts at Increasing Age of Parabiotic Union

Pairs of Parabionts	Days of Parabiosis	Evidence of Cross Circulation			
			Method		
		PSP	Evans Blue	India Ink	Results
1	3	+	..	..	Positive
12	4	+	..	+	Positive
3*	4	+	..	..	Negative
1	4	..	+	+	Positive
2	5	+	..	+	Positive
2	6	+	..	+	Positive
1	7	+	..	+	Positive
5	7	..	+	+	Positive
5	7	..	+	+	Negative
1	9	+	..	..	Negative
4	10	+	..	+	Negative
2	11	+	..	+	Negative
1	11	..	+	+	Negative
1	12	+	..	+	Negative
1	13	..	+	+	Positive
7	13	..	+	+	Negative
1	14	+	..	+	Negative
1	16	+	..	+	Negative
1	17	+	..	+	Negative
2	18	+	..	+	Negative
1	18	..	+	+	Negative
6	20	+	..	+	Negative
8	20	..	+	+	Negative

* Hematomas at parabiotic junction.

The same method of anastomosis was used in uniting the ears of a single animal to provide control autologous junctions for comparison with the homologous parabiotic junctions.

Attempts to demonstrate cross circulation at varying durations of parabiosis were made in the following manner: As a physiological means of demonstrating continuity of circulation, two dyes were employed. At a specified duration of parabiosis, 1 ml. of phenolsulfonphthalein (PSP) (6 mg. per milliliter) was injected intravenously into one parabiont. One to two hours later the apposing parabiont was catheterized and the urine examined for PSP. Some animals were injected on several occasions during the period of parabiosis.

Evans blue dye (T-1824) was similarly employed. A 0.3% solution (8 ml.) was injected intravenously into one parabiont after plasma had been drawn and standards established with a known quantity of the dye in a Beckman spectrophotometer. Plasma samples were withdrawn from the parabionts 1 hour and 24 hours later. The amount of dye per milliliter of plasma was then determined spectrophotometrically.

After completion of the final dye studies, India ink diluted with two volumes of isotonic saline and warmed to 39 C. was used to fill the aural vasculature. Since the aural vasculature of the rabbit has many arteriovenous shunts, the venous route of injection of ink was found most

practicable.[3] The marginal aural vein of one parabiont was cannulated against the blood flow, and by means of a two-way Luer syringe as much blood as possible was cleared from the aural vasculature by the injection of isotonic saline. Then 8 ml. of diluted India ink was injected. Immediately after the injection the bases of the ears proximal to the level of surgical union were simultaneously clamped transversely so as to prevent continued escape of India ink into the general circulation. The animals were then killed by injection of procaine into the cisterna magna. After excision of the ears, with clamps intact, the animals were weighed and complete autopsies done. The ears were placed in 10% formalin. After fixation, celloidin sections, cut in a

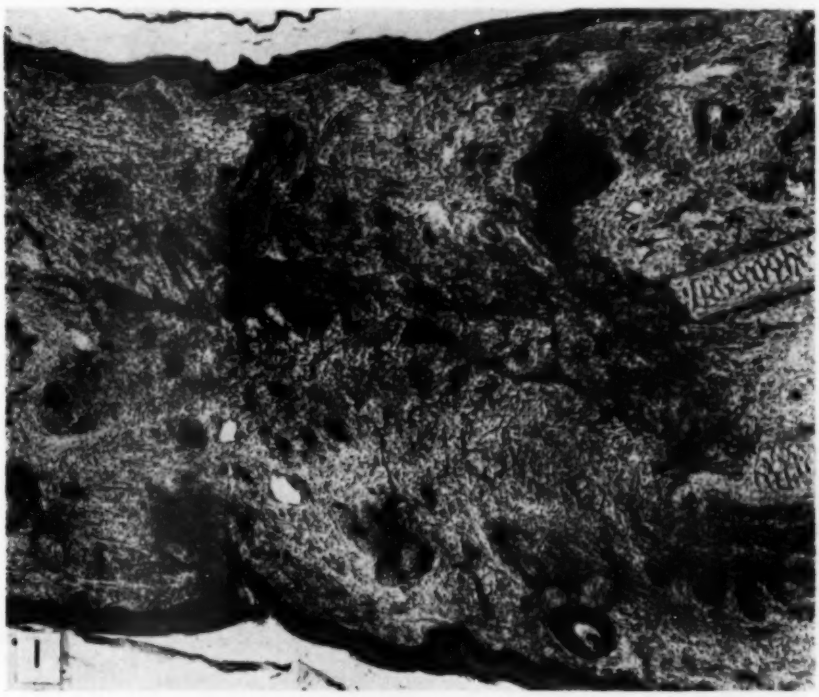


Fig. 1.—Medium-power photomicrograph of an homologous aural junction 4 days of age. Note the good approximation of homologous tissues at this age of union, with exception of the area between the cartilages of the two animals, shown on the right of the illustration. This space contains new granulation tissue. Midway between the epithelial surfaces lies the zone of apposition of homologous tissues. This contains many capillaries filled with India ink. The tissues of the upper animal were injected with India ink. Vascular channels of the lower animal also contain ink, indicating that continuity of circulation between rabbit parabionts was fully established at this age of healing. × 40.

transverse plane perpendicular to the aural junction and stained with hematoxylin and eosin, were prepared for routine microscopic study. Special stains for collagen and reticulum were done when indicated.

Autologous aural junctions were similarly injected with India ink and prepared for microscopic study.

Other organs and tissues were fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin for microscopic study in search for evidence of systemic effects of parabiosis.

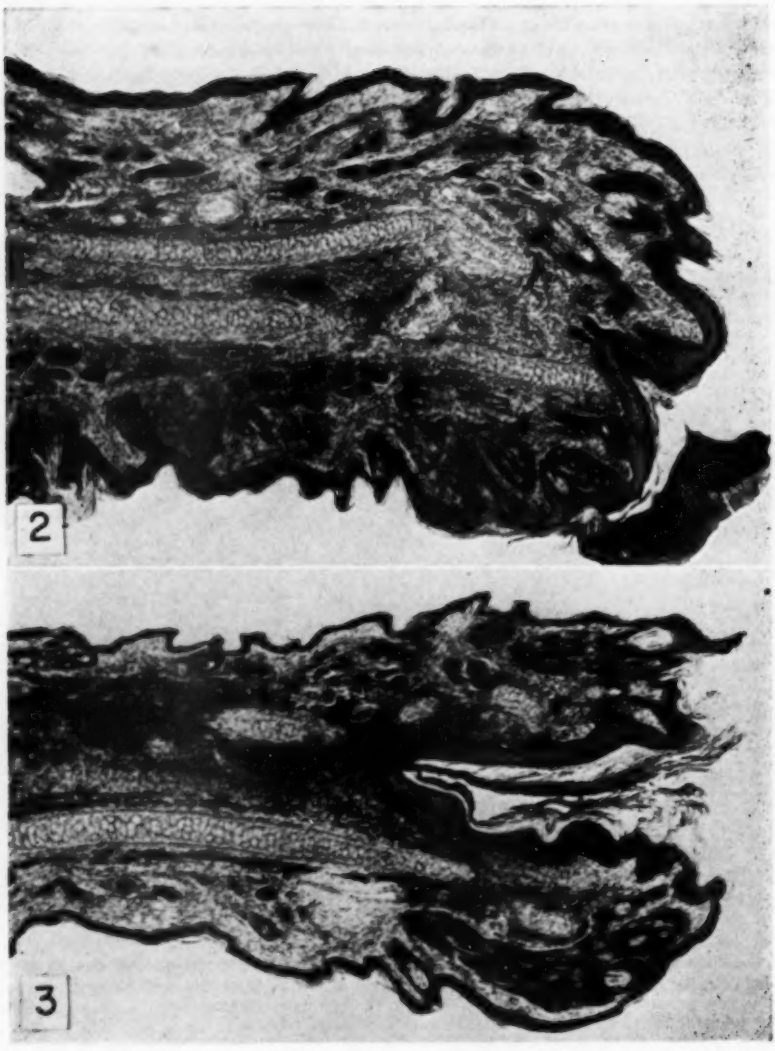


Fig. 2.—Low-power photomicrograph of an homologous aural junction one week of age. Cartilage in the upper half of the illustration belongs to one parabiont. The lower cartilage belongs to the opposite parabiont. Note the well-healed appositional zone between the cartilages. This zone shows an early lymphocytic infiltration. The epithelial surfaces are well healed and in perfect continuity. Cross circulation was still demonstrable in about half the experiments at this age of union. × 20.

Fig. 3.—Low-power photomicrograph of an homologous aural junction 3 weeks of age. Note the fissure which has developed between the epithelial surfaces of the two parabionts. The base of fissure is continuous with the more or less linear inflammatory incompatibility reaction which traverses the entire length of the section along the homologous junction. Circulation between parabionts was never demonstrated at this age of union. × 20.

RESULTS

Ninety pairs of rabbits were placed in parabiosis for varying periods of time. The mortality rate was 46%, most animals dying between the 9th and 18th days of parabiosis. This figure is similar to the mortality reported for parabiotic mice and rats.

Demonstration of Temporary Cross-Circulation by Dyes.—The injection of phenolsulfonphthalein into one member of 21 pairs during the first week of parabiosis showed 18 pairs with a cross circulation and 3 pairs without a cross circulation (Table). The absence of a cross circulation in three pairs seemed to be due to hematomas which separated the tissues of the parabionte at the site of anastomosis. Among 10 pairs of animals in which one parabiont was injected with Evans blue on the seventh day of parabiosis, a cross circulation was demonstrated in only 5 pairs. Equilibration of the dye between parabionts occurred within 24 hours after injection. One pair injected at four days showed cross circulation of the dye, confirming the usual experience with PSP.

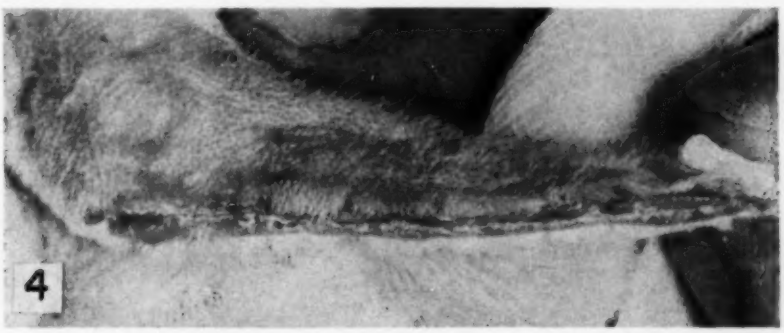
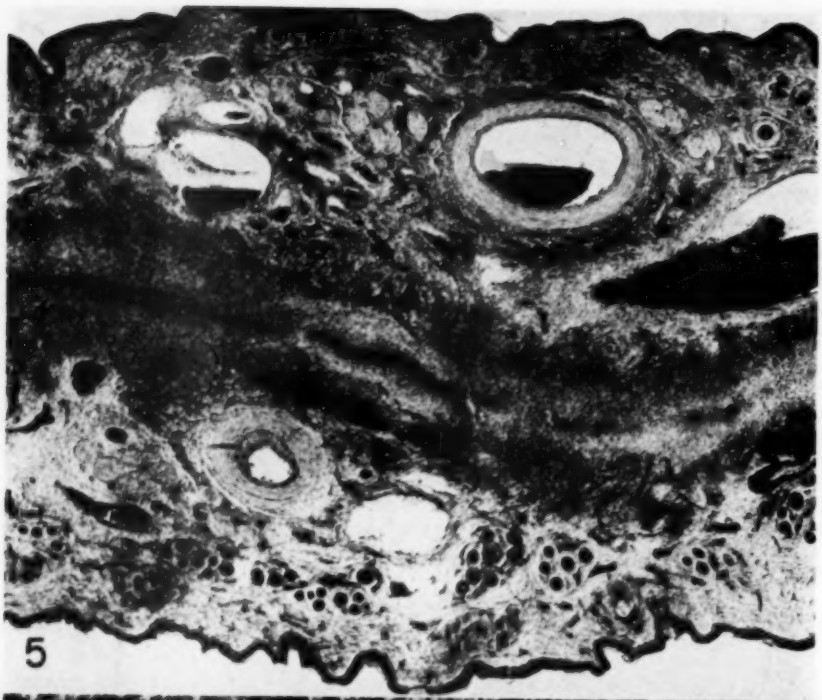


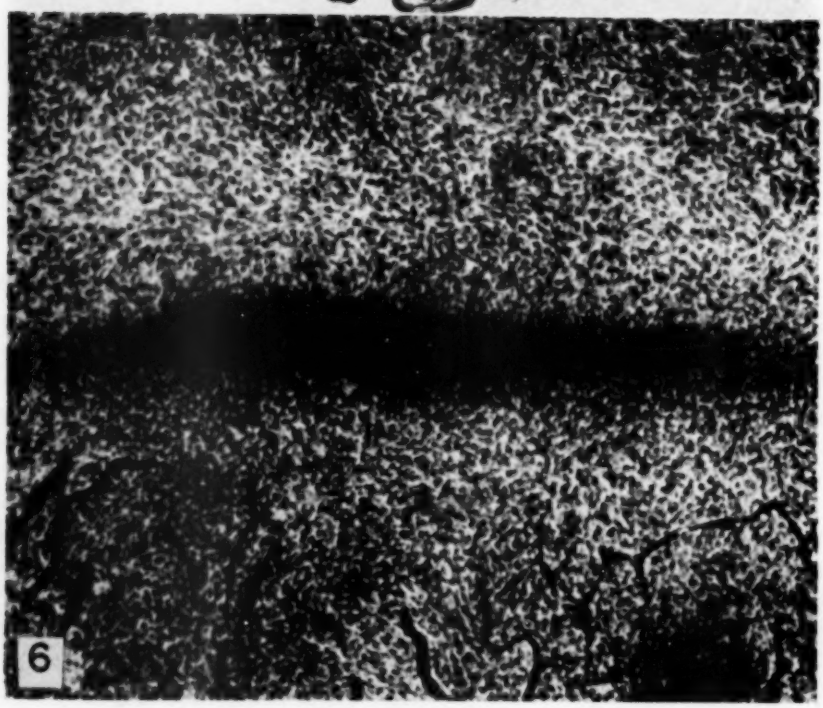
Fig. 4.—Margin of an homologous aural junction 3 weeks of age. Note the absence of edema or other evidence of active inflammation and fact that the apposing tissues remain in good approximation. In the transverse axis of the illustration, the narrow epithelial fissure between the apposing tissues of the two animals is barely distinguishable. Natural size.

The studies with PSP in nine pairs of animals during the second week of parabiosis showed no cross circulation (Table). Of these, seven pairs had previously been injected with PSP and showed cross circulation at four days. Among nine pairs of animals in which Evans blue was employed late in the second week of parabiosis, only one pair showed a cross circulation. One pair of the group with no cross circulation had a cross circulation demonstrated with PSP after four days in parabiosis.

The studies during the third week of parabiosis, using PSP and Evans blue in 19 pairs of animals, showed no cross circulation (Table). Seven pairs which showed cross circulation with PSP at four days of parabiosis exhibited no cross circulation on subsequent study during the third week.

The demonstration of the presence or absence of a cross circulation in 64 pairs of rabbits by use of circulating dyes was confirmed microscopically by the presence or absence of India ink in the vasculature of the uninjected parabiont.





Figures 5 and 6

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In single animals in which one ear had been completely transferred to the apposing ear, the injection of Evans blue into the systemic circulation resulted in almost immediate discoloration of the transplanted ear.[2]

Gross Studies of Autologous and Homologous Junctions.—Autologous and homologous junctions at the end of seven days were well healed and in good approximation. The edema and turgor which developed subsequent to the operative procedure had subsided in most instances. The accumulation of conspicuous amounts of blood or serum between the united ears was seldom encountered. Dermal epithelium was continuous. At the end of the second week the two types of junctions remained grossly similar except that the homologous aural junction exhibited a fissure at the site of previous healing of dermal epithelium of the parabionts along the line of surgical approximation (Figs. 3 and 4). Both types of junctions showed normal cutaneous color and regrowth of hair. By the end of the third week, the autologous aural junctions were still firmly healed, and the epithelial surfaces at the site of anastomosis remained in continuity. On the other hand, the fissure separating the epithelium of the parabionts at the homologous aural junctions appeared to deepen (Fig. 3). A scaly exudate formed continuously at the pit of the fissure. Otherwise, the homologous aural junctions at 3 weeks of age remained free of edema or gross signs of active inflammation and were in good approximation (Fig. 4). There were no conspicuous progressive changes at junctions of tissues of animals maintained in parabiosis beyond three weeks up to as long as four and one-half months.

Microscopic Studies of Autologous and Homologous Junctions.—Microscopic study showed that the tissues placed in approximation consisted of dermal epithelium, underlying collagenous tissues, and cartilage (Figs. 1, 2, 3, and 5). The resected margins of dermal epithelium of the animals were adjacent to each other. The peripheral rim of cartilage and perichondrium of the two ears were also in direct apposition. The principal remaining area of contact between animals consisted of the connective tissues, with irregular shreds of perichondrium

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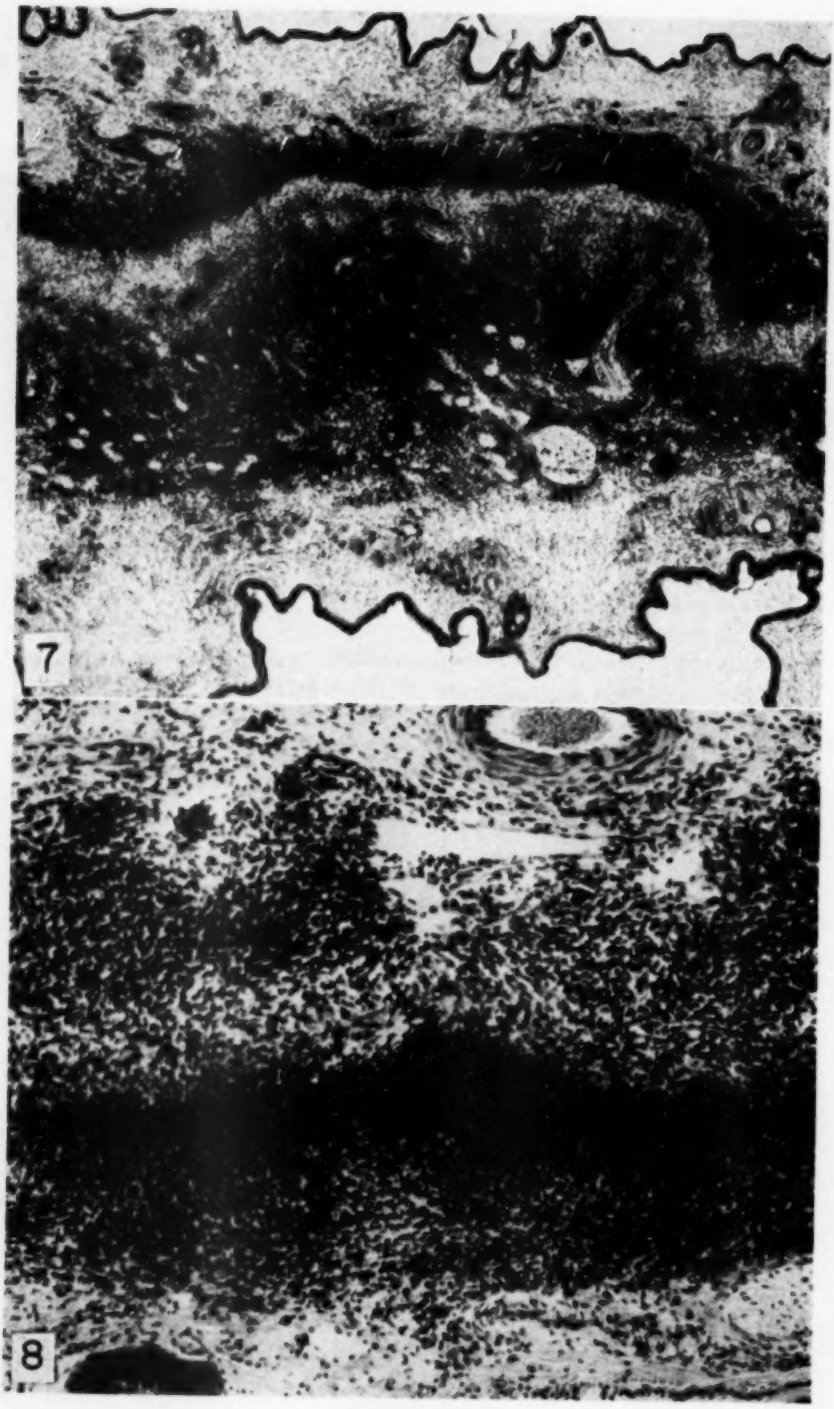
EXPLANATION OF FIGURES 5 AND 6

Fig. 5.—Medium-power photomicrograph of the central area of an homologous aural junction 3 weeks of age. The epithelial surfaces are normal. The subepithelial tissues in the upper half of the illustration are involved in a diffuse inflammatory reaction. In the midtransverse axis there is a well-demarcated linear inflammatory reaction. The parabionts contributed equally to this reaction, so that it was designated as a symmetrical type. The dark linear interrupted areas at the immediate zone of apposition represent foci of cellular necrosis between homologous tissues. The central artery in the lower half of the illustration shows a type of intimal proliferation which was commonly encountered in homologous aural junctions at about 3 weeks of age. The vasculature of the tissues of the parabiont occupying the lower half of the illustration was injected with India ink. Note that the ink did not cross the junction into the vessels of the uninjected parabiont. Cross circulation was never demonstrated at this age of union. × 40.

Fig. 6.—High-power photomicrograph of the linear appositional homologous reaction shown in Figure 5. The zone of immediate contact of homologous tissues consists of a plane of severe cellular necrosis, which lies in the midtransverse axis of the illustration. On each side of the zone of apposition there is a very narrow zone containing polymorphonuclear leucocytes and a few macrophages. Adjacent to this on each side is a broad pale zone which contains a preponderance of macrophages. At the extreme upper and lower margins of the illustration lie the zones of heavy lymphocytic infiltration. As shown by capillaries injected with ink in the lower half of the illustration, continuity of circulation between parabionts was not demonstrable at this age of union. The capillaries rarely entered the macrophage zone and appeared to end predominantly in the lymphocytic zone. × 250.



Figures 7 and 8

(See legends on opposite page)

stripped from resected cartilage intermittently dispersed along the plane of surgical apposition. The central arteries were usually opposite each other on the two sides of the junction.

Forty autologous junctions between 1 and 3 weeks of age were studied microscopically. At the end of the first week the junctions showed complete and uneventful healing.[2] The tissues were in good approximation, and healing was so perfect that it was not possible to distinguish between tissues of the two ears at the zone of apposition. Along the central plane of apposition there was little deposition of new collagen and reticulum. The regions of greatest fibroblastic activity were in the intercartilaginous areas, where considerable granulation tissue was usually formed. Here, also, the formation of new capillaries was most conspicuous, being especially well shown in the preparations injected with India ink. There were surprisingly few vascular connections demonstrable in serial sections across the line of apposition of the autologous junctions, even though gross studies had indicated a rich cross circulation. Larger vascular channels on either side of the zone of apposition were filled with India ink in a manner which indicated the presence of arteriovenous shunts. The dermal epithelium of the apposing tissues was continuous, so that distinction between epithelium of one ear and that of the opposite ear of the same animal was not possible. The tissues were free of inflammation with the exception of a mild reaction around hair follicles.

Sixty-four homologous junctions were studied microscopically. Of these, 13 were 4 days of age. These showed good approximation of tissues of the parabionts. The epithelial surfaces were in continuity, the surgical defect having been bridged by several layers of squamous cells, arising apparently from the dermal epithelium of both parabionts. The intercartilaginous areas were occupied by rapidly proliferating granulation tissue. In these areas, especially, capillaries distended with India ink were traced across the plane of tissue anastomosis, so that it was concluded that the circulation between parabionts was conducted principally through these channels (Fig. 1). Along the plane of collagenous junction a few lymphocytes had accumulated around small vessels, but fibroblastic and collagenous healing seemed to have proceeded normally, without conspicuous formation of new vascular networks.

EXPLANATION OF FIGURES 7 AND 8

Fig. 7.—Medium-power photomicrograph of an homologous junction 3 weeks of age. In the transverse axis of the illustration there is a broad inflammatory zone which, in this instance, shows asymmetry of cellular response and vascular disease. The tissues of the upper animal, the "dominant" reactor, show a well-defined lymphocytic zone. At the lowermost margin of this zone there is a narrow zone of appositional necrosis, which is not well shown at this magnification. Below this there is a broad pale zone consisting largely of monocytes infiltrating the tissues of the opposite parabiont, the "weak" reactor. This zone merges with a broad diffuse intense inflammatory reaction with conspicuous edema and angiitis. × 40.

Fig. 8.—High-power photomicrograph of an homologous aural junction 3 weeks of age. In the midtransverse axis lies the zone of apposition, which consists of cellular necrosis. The tissues of the lower animal show an intense mixed infiltration with inflammatory cells which have undergone acute necrosis. The tissues of the upper animal show narrow monocytic and well-demarcated lymphocytic zones. This response is asymmetrical, with the "dominant" reactor in the upper and the "weak" reactor in the lower part of the illustration. It typifies the individual variations encountered when the tissues of one animal were placed in contact with those of another of the same species and strain. × 250.

Figures 9 and 10

(See legends on opposite page)

Fifty-one homologous junctions between 1 and 3 weeks of age were studied microscopically. The junctions at the end of the first week showed epithelial continuity, good approximation of collagenous tissues, and infiltration of inflammatory cells in the zone of apposition (Fig. 2). The cellular infiltration consisted principally of local accumulations of lymphocytes between vascular channels. Active healing was still in progress between the cartilaginous bars, particularly those cephalad in location. This consisted of an ingrowth of delicate granulation tissue from one or both animals. India ink, when used, was easily recognized in vascular channels on both sides of the zone of apposition in animals which had a cross circulation demonstrated with PSP or Evans blue. At homologous junctions in which the inflammatory reaction was more advanced, a cross circulation was not demonstrated either with the dyes or with India ink. In these instances the dermal epithelium was beginning to deteriorate at the immediate line of junction. This change was always associated with the spread of a conspicuous stromal and inflammatory incompatibility reaction to the basal layer of the epithelium, with infiltration of the stratified squamous epithelium by inflammatory cells. The dermal epithelial deterioration at the junction was apparently secondary to the subjacent stromal inflammatory reaction.

During the second week, and the time thereafter, the autologous aural junctions remained completely healed without a trace of inflammation. At 7 to 14 days of age the homologous aural junctions showed rapid progression of the inflammatory reaction at the zone of apposition. The reaction now occupied about one-fourth of the width of the celloidin section and usually bracketed the extent of the stromal appositional zone from the pit of one epithelial fissure to the other. The exudate from the homologous granulation tissues partly filled the fissures with serum and necrotic inflammatory cells. Subjacent to the fissures and from either side, the migration of polymorphonuclear leucocytes and monocytes was conspicuous. Acute angiitis and endarteritis of vessels within and near the zone of apposition were now more pronounced. The capillaries filled with India ink appeared to terminate in

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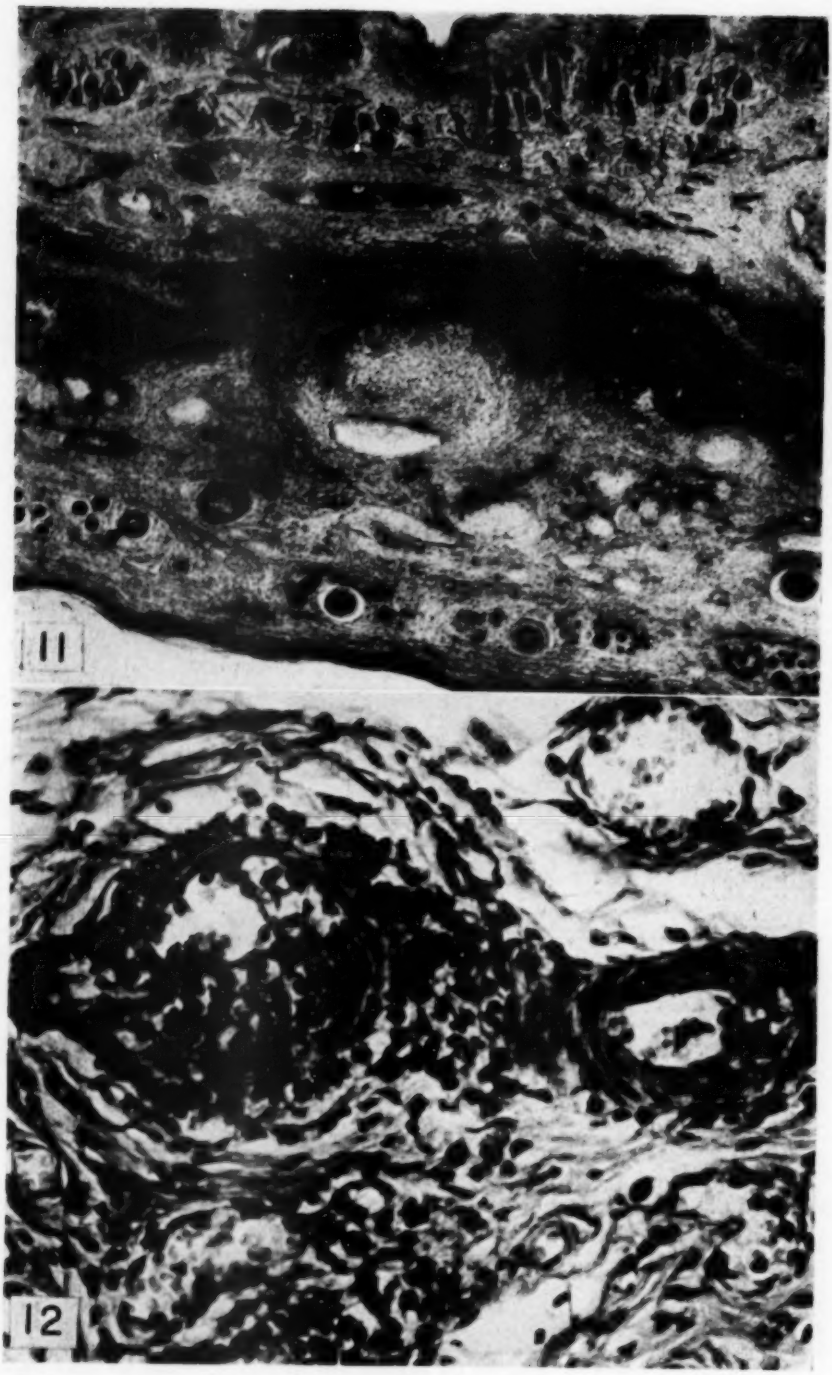
EXPLANATION OF FIGURES 9 AND 10

Fig. 9.—Medium-power photomicrograph of an homologous aural junction 3 weeks of age. This shows one type of healing which occurred between the peripheral rims of cartilage of the two animals. In the right half of the illustration the inflammatory reaction which occurred between homologous tissues is shown. The zone of necrosis ends at the tip of the uppermost bar of cartilage. Between the cartilages of the two animals in the left half of the illustration perfect fibrous healing has taken place. This region has been healed solely by ingrowth of granulation tissue from the lower animal. The cartilage and the perichondrium of the upper animal show no adverse effects due to contact with homologous granulation tissue. The vasculature is completely filled with India ink, but none of the injected ink passed across the junction into the vessels of the opposite parabiont, whose tissues occupy the lower half of the illustration. × 40.

Fig. 10.—Medium-power photomicrograph of an homologous junction 3 weeks of age. This illustration shows another type of healing between the cartilages of the parabionts. The intercartilaginous zone has been healed by ingrowth of granulation tissue of both animals so as to produce three distinct zones of inflammation at points of contact. The tissues of the upper animal have been injected with ink. Ink-filled vessels can be traced into the intercartilaginous zone, where they appear to terminate in the region of the inflammatory response. This illustration shows the remarkable specificity of the inflammatory reaction which occurred wherever newly formed vascularized connective tissues of the apposing animals were in contact for a few days. × 40.



Figures 11 and 12

(See legends on opposite page)

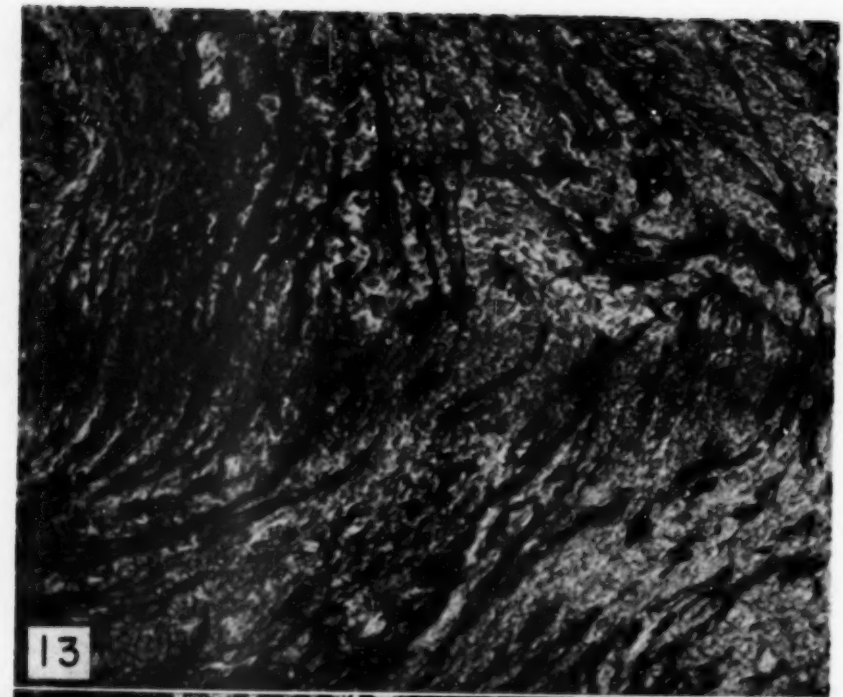
the inflammatory zone on the side of injection. Continuity of circulation between animals was not demonstrated by detection of capillaries traversing the junction or distended with ink on both sides of the junction.

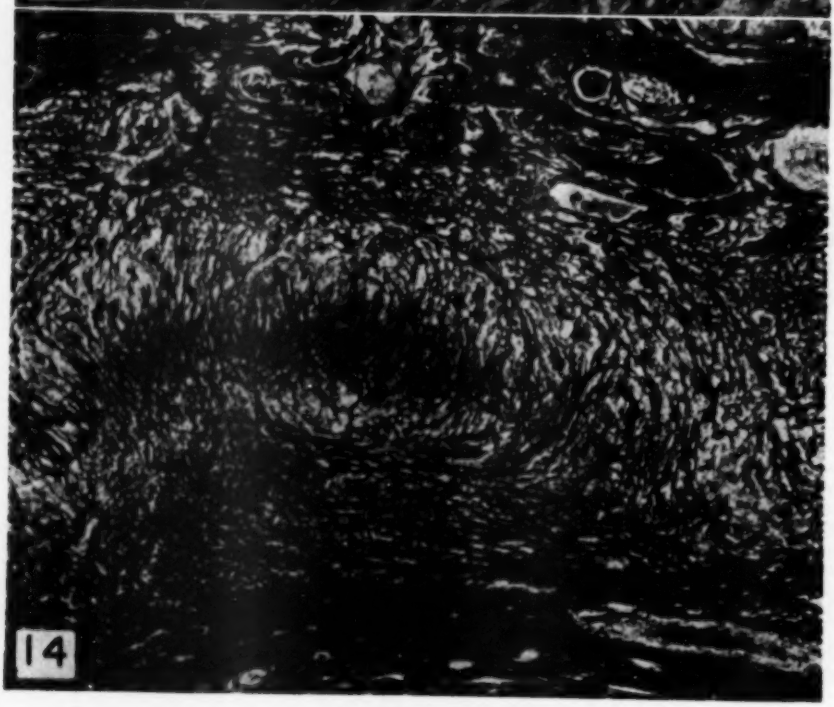
By the end of the third week, at homologous junctions the inflammatory reaction had reached its maximum. It occupied about one-third of the breadth of the celloidin section (Fig. 5). It was a linear orderly inflammation which was usually composed symmetrically of several well-developed strata on each side of the junction (Fig. 6). The immediate zone of apposition contained focal areas of cellular necrosis with a matrix of acidophilic ground substance, the exact nature of which was undetermined but which remotely resembled "fibrinoid" material (Fig. 6). To each side of the central zone of necrosis, migrating polymorphonuclear leucocytes and a few monocytes were situated. Lateral to these was a broad pale zone of monocytes with a few lymphocytes and polymorphonuclear leucocytes. External to this there was a broad zone which was compactly infiltrated with lymphocytes. Capillaries filled with India ink appeared to terminate in this lymphocytic zone and rarely penetrated the monocytic zone, which was therefore essentially avascular. In the less dense areas of infiltration within the outermost lymphocytic zone, angiitis and endarteritis were often conspicuous. Small vessels filled with leucocytes and proliferating endothelial cells without occluding fibrinous thrombi were notable. Frequently, the central arteries had eccenteric areas of intimal proliferation located at the side of the lumen adjacent to the inflammatory reaction and the appositional zone (Fig. 5). Involvement of vessels external to the inflammatory zone occurred only in junctions 3 weeks of age or older (Fig. 12). The angiitis, however, did not spread to the systemic circulation in a way recognizable by routine microscopic study of organs. The epithelial fissures had deepened so as to extend down to the margin of the cartilaginous bar of one or the other parabiont. Within the intercartilaginous areas two types of healing were encountered. In one type, the area was healed in completely by the granulation tissue of a single animal (Fig. 9). In the other type, the granulation tissues of the two animals shared in the process, so that foci of inflammation developed wherever the vascularized homologous granulation tissues met (Fig. 10). These areas of inflammation were not stratified like those along the general line of apposition, presumably because of the briefer period of contact of the homologous granulation tissues. In places where cartilage

EXPLANATION OF FIGURES 11 AND 12

Fig. 11.—Medium-power photomicrograph of an homologous aural junction 3 weeks of age. In the transverse axis of the illustration lies the intense local inflammatory reaction on each side of the junction of apposing homologous tissues. In central location and just below the junction there is an artery which has undergone reorganization subsequent to thrombosis. The interior of the vessel and its wall are filled with granulation tissue arising from both animals. Where the vascularized granulation tissues of the two animals meet, there is a characteristic inflammatory reaction, which in this instance traverses the vessel wall and mural thrombus. The vessels of the upper animal have been injected with India ink. Capillaries filled with ink can be seen superior to the zone of necrosis within the vessel wall, but there is no evidence of cross circulation. × 40.

Fig. 12.—High-power photomicrograph of an area adjacent to the inflammatory reaction between homologous tissues at 3 weeks of age. Several vessels show perivascular infiltration with lymphocytes and endothelial proliferation. Early involvement of small vascular channels is characteristic of the homologous response and the angiitis usually increases in proportion to the age of union over a period of about three weeks. × 450.





Figures 13 and 14

(See legends on opposite page)

was immediately adjacent to homologous granulation tissue, no degenerative changes were noted in the cartilage. In other areas, where cartilage was adjacent to autologous granulation tissues, foci of proliferation of cartilage cells were common.

It seemed curious that the extensive necrosis, inflammation, and interruption of capillary circulation did not lead to a separation of the ears along the plane of apposition of the ageing homologous tissues. In spite of the intensity of the fully developed reaction, collagenous and reticular fibers which had been laid down across the junction during the first few days of healing persisted and maintained good approximation of the tissues (Figs. 13 and 14). For this reason the tissues of the two animals were permanently and firmly united, at least for the duration, four and one-half months, of the longest experiment. This union was especially impressive when attempts were made to pull them or dissect them apart.

Relations Between Asymmetric Reactions at Parabiotic Junctions and Systemic Pathologic Changes.— Of 51 homologous aural junctions one week of age and older, 39 were classified as being "symmetrical" on the basis of equal inflammatory responses on the two sides of the appositional zone. The remaining 12 were classified as "asymmetrical" because of the inequality of the inflammatory responses on the two sides of the junctions (Figs. 7 and 8). One side of the "asymmetrical junction" showed a well-developed zone of lymphocytic infiltration characteristic of the "dominant reactor." This blended into a definite monocytic zone, which, in turn, joined a zone of scattered polymorphonuclear leucocytes adjacent to the necrotic appositional zone. In the aural tissues of the "weak reactor," cellular necrosis and inflammation were broadly distributed and less demarcated. The inflammatory response appeared even to involve the subepithelial tissues, and angiitis was more prevalent than in the tissues of the "dominant reactor." Epithelial discontinuity and intercartilaginous healing occurred in much the same way as in homologous junctions with "symmetrical reactions." The animals were weighed prior to parabiosis and again at death. All animals lost weight while in parabiosis, but those which died spontaneously lost more than the average. Among the 39 pairs classed as "symmetrical reactors," there was a spontaneous mortality of 1 animal in each of the 13 pairs. Ten of the 12 pairs classified as "asymmetrical reactors" showed a spontaneous mortality of one animal in each pair.

The microscopic study of organs of the animals failed to reveal consistent pathologic changes which might be interpreted as due to a generalized systemic

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EXPLANATION OF FIGURES 13 AND 14

Fig. 13.—High-power photomicrograph of an homologous aural junction 3 weeks of age. (Mallory's aniline blue connective tissue stain). The inflammatory zone between homologous tissues lies within the midtransverse axis of the illustration. Darkly stained bundles of collagenous fibers cross the junction in various directions, thus maintaining good approximation of the apposing tissues. The collagen seems to be derived from fibroblasts of both animals. It is curious that it was not destroyed by the incompatibility reaction. × 300.

Fig. 14.—Medium-power photomicrograph of an homologous aural junction 3 weeks of age. (Foote's reticulum stain). The inflammatory reaction between homologous tissues lies within the midtransverse axis of the illustration. Note that the tissues of the two animals remain in good approximation, despite the linear zone of junctional necrosis. The adherence of the homologous tissues to each other is to be attributed to the more or less perpendicularly arranged bundles of reticulum and collagen which traverse the junction and maintain a very firm union. Collagen and reticulum, presumably formed by fibroblasts of both animals, were resistant to the incompatibility reaction. × 40.

reaction induced by the parabiotic state. Varying degrees of pneumonitis, encephalitis, gastroenteritis, and coccidioidal hepatitis were encountered, especially among animals which died spontaneously. There was no generalized arteritis or other form of significant vascular disease. Nine animals had an unexplained atrophic bone marrow, which is now being more thoroughly studied. Two of these showed "asymmetrical" reactions at the aural junctions and died spontaneously. Among the remainder, which had "symmetrical" reactions, four died spontaneously and three were killed.

COMMENT

The aural method of parabiosis in rabbits was used for several reasons. First, the granulating wounds of normal tissues of two animals of the same species and strain with independent blood supplies could be placed in continuous approximation for varying periods of time. The anatomical features of the surgical union viewed in microscopic slides permitted sharp differentiation between the tissues of the apposing animals. Physiological and anatomical circulatory studies could be made between parabionts with facility. The technique of anastomosis could be applied to two ears of a single animal to provide control autologous material. The healing processes between autologous and homologous aural junctions of various ages could be studied without encountering complications of the customary abdominoperitoneal techniques of uniting two animals.

It has been generally assumed that parabiotic union of members of the same species of common small experimental animals leads to the development and persistence of a cross circulation. Proof for this was difficult to find in the literature, even though the validity of conclusions of many reported parabiotic experiments depended upon such an assumption. Duschl and Niekau attempted to prove the presence of a cross circulation by direct low-power microscopic inspection of the shaved skin at abdominoperitoneal junctions between living rats.[4] They described vessels passing across the anemic scar at about 10 days of parabiosis. Trypan blue injected into one rat crossed to the parietal peritoneum of the opposite parabiont in about half an hour, and the animals equilibrated the dye in about 15 hours. Clear proof of vascular connections between parabionts was not given. Cristea and Denk injected rabbit parabionts (10 to 12 days after surgical union) with hirudin intravenously and found a greatly prolonged clotting time in the injected parabiont but no change in the clotting time of the partner.[5] This observation was presented as evidence against the presence of a cross circulation. More recently, Hill injected parabiotic rats with vital red and found 20% of the dye in the uninjected parabiont after one hour.[6] He concluded that the vascular connections were of capillary nature but failed to demonstrate the connections. Huff, Trautman, and Van Dyke injected Fe^{59}-tagged rat erythrocytes into inbred strains of rats which had been in parabiosis from one to six months and found that 0.66% of the blood volume per minute was transferred from one animal to the other.[7] Microscopic definition of the circulation between parabionts was not attempted. Such incomplete evidence as there is indicates that persistence of a cross circulation may occur when parabionts belong to the same highly inbred strain. When less highly inbred animals were used, there was no agreement in

the literature as to whether the transport of materials, especially dyes, occurred at all between parabionts. If transport was demonstrated, the question as to whether transport was by blood, lymph, or peritoneal fluid remained unanswered.

The present studies have shown that continuity of circulation was temporarily established between rabbit parabionts which were not highly inbred and that the vascular connections were primarily capillary in nature. The cross circulation was well developed by the third or fourth day of parabiosis and persisted on the average for about seven days. The temporary nature of the cross circulation and the mechanism of its destruction seemed to be related to the progressive development of a characteristic inflammatory reaction at the junction of tissues of the parabionts.

These studies also proved that autologous and homologous aural junctions showed similar initial sequences of healing, with the early establishment of fibroblastic collagenous union, dermal epithelial continuity, and cross circulation through capillary anastomoses. Despite this apparent early stage of mutual compatibility of parabionts, traces of a developing incompatibility tissue reaction were recognizable as early as the fourth day of surgical union. As this reaction developed, after an average of seven days, the circulation between parabionts was diminished or interrupted as a characteristic form of inflammation intervened along the plane of healing at the surgical union. Epithelial fissures with discontinuity then developed at the homologous junction, due presumably to subjacent stromal reactions rather than to incompatibility of the previously fused contiguous layers of epithelium of the two animals. Angiitis adjacent to the junction progressed in rough proportion to the age of union. During early stages of healing, collagenous and reticular fibers, presumably arising from fibroblastic activity of both animals, bridged the junction and maintained good approximation of the tissues of the parabionts despite the intense inflammatory incompatibility reaction and the progressive loss of cross-circulation. The incompatibility reaction, severe though it was and essentially destructive, apparently was not directed against the newly formed fibrillary intercellular materials. Collagen and reticulum of one animal were well tolerated by another animal of the same species and strain for many weeks under the circumstances described. This conclusion was conditioned by the assumption that the newly formed collagen and reticulum were individual, and not mutual, products.

The reaction of one animal's tissues to those of another in the presence of individual intact blood supplies resulted in the development of a type of inflammation which was similar to the unilateral host reaction to free musculofascial homografts.[1] The specificity of the inflammatory reaction as a consistently reproducible and uniform response was notable. It was encountered wherever vascularized homologous granulation tissues met—between the cartilages, within organizing thrombosed vessels (Fig. 11), and along the general plane of apposition of collagenous tissue. The reaction terminated in the production of a chronic granuloma which was different in some respects from granulomatous inflammations of common pathologic processes. The immediate zone of contact between homologous tissues consisted of irregularly dispersed foci of cellular necrosis. Giant cell formation, conspicuous phagocytic activity, and hemosiderin deposition were not encountered. A continuous migration of leucocytes in the direction of the zone of apposition was apparent despite the decrease in vasculature across the junction as it increased in

age. Angiitis and endarteritis contributed to the occlusion of vascular channels and interruption of cross circulation. There was no tendency toward progressive deposition of collagen and reticulum beyond the amounts accumulated during the first week of union. During the third week, the reaction retained essentially its fully developed character, and it neither increased nor decreased in amount or intensity with further passage of time.

The ability of homologous tissues of rabbits in parabiosis initially to heal together at several tissue levels was impressive. Early in the healing process, there was union at endothelial, fibroblastic, collagenous, and epithelial levels. A capillary circulation, though transitory, was promptly established between the parabionts. Proliferating fibroblasts of both animals led to deposition of collagen and reticulum which bridged the plane of surgical anastomosis and united the interstitial tissues of the two animals. The dermal epithelial surfaces were intimately healed together, with no distinction between the cells of the parabionts at the line of anastomosis. The initial cellular activity closely resembled the healing of autologous tissues, with the possible exception of the coincidental development of the earliest trace of the incompatibility reaction. The sequence of healing at homologous aural junctions was similar in some respects to the sequence described by Rous in organization by the host of embryonic tissues upon transplantation.[8] Rous showed that homologous transplants of mouse embryonic tissues were generally capable of growth in the new host for a period of 7 to 10 days. During this time there was proliferation of connective tissues, cartilage, and dermal epithelium of the embryonic explant. Capillary union with the host's tissues occurred. After the brief period of cellular activity, the explants regressed and were generally absorbed.

Vascular injury at homologous aural junctions was a conspicuous component of the incompatibility reaction. Early involvement of the vasculature was confined to small channels within the immediate vicinity of the zone of apposition. The exact method of interruption of the cross circulation was not determined as a well-defined series of histopathologic sequences. Capillaries simply disappeared from the junctional zone as the incompatibility reaction increased in density. Fibrinous thrombi were seldom encountered. Swollen endothelium, macrophages, and polymorphonuclear leucocytes seemed to be the principal elements which occluded the lumina of small vessels. In spite of the intense inflammation and the progressive loss of circulation across the plane of tissue anastomosis, the development of more numerous vascular channels to the vicinity did not occur. After two weeks or more of parabiosis, larger arteries and veins in the vicinity of the reaction were involved, so that panarteritis and panphlebitis were often conspicuous. The vascular injury remained confined in the neighborhood of regions of contact of homologous tissues and was never generalized throughout other, more distal organs and tissues of the parabionts. In this respect the types and variations of vascular disease were similar to those observed in a study of homologous musculofascial transplants, wherein only the vessels of the host involved in organization of the transplant were affected.[1]

It should not be inferred from the preceding comments that all incompatibility reactions of the same age were identical. There were differences in the over-all reactions among a series of parabiotic pairs, but these were principally differences in degree rather than basic pattern of reactions. Also, the reactions of two members of a pair were not always identical. When the difference in the reaction of

two members of a pair was considerable, the junctional reaction was designated as an asymmetrical one. In such instances one member of the pair reacted in the usual way and was designated as the "dominant" reactor. The opposite member of the pair ordinarily reacted with a less well-defined inflammatory barrier and a more pronounced angiitis. This member was designated as the "weak" reactor. Though a careful survey has been made of the clinical records and postmortem findings in these cases, we have no explanation for the observed differences in reactions. Nor was there any explanation for similar differences in reactions when the objects of study were free musculofascial homografts.[1] Perhaps these matters are related to the state of "parabiotic intoxication" which is so often emphasized as a cause of illness and death in parabionts. If there is an intoxication due to parabiotic union in rabbits, the present experiments and those of Beer have failed to disclose a consistent pattern of systemic histopathologic changes which can be correlated with the intoxication.* Doubtless more refined methods must be used before these problems can be solved.

SUMMARY

Rabbits of the same strain, but not highly inbred, were united in parabiosis by surgical anastomosis of their ears. During the first few days the tissues of the parabionts healed together almost as perfectly as comparable tissues of single animals in the control experiments. Mutual interpenetration of granulation tissue of the two animals was sufficient to establish a cross circulation and a firm collagenous union across the plane of anastomosis within three or four days. Even the dermal epithelium of the two animals grew together to bridge the united granulation tissues with a continuous multilayered epithelial surface.

As the healing progressed to completion, it had to compete with an inflammatory reaction which developed with gradually increasing intensity on each side of the zone of contact of the granulation tissue of the parabionts. This reaction, which was ordinarily of equal magnitude on the two sides of the zone of contact, usually reached its maximum intensity within two or three weeks and remained unchanged thereafter. The first evidence of the reaction was a mild angiitis, which was detectable in the zone of apposition of the homologous granulation tissues as early as the third or fourth day. Within the next few days, the progressive angiitis was obscured by the development of linear interrupted areas of acute necrosis in the appositional zone. This process was accompanied by a conspicuous marginal accumulation of polymorphonuclear leucocytes, external to which there was a broad granulomatous zone of monocytes and lymphocytes with numerous vascular channels showing various degrees of angiitis.

The gradual development of this incompatibility reaction was accompanied by a partial reversal of the healing process, for during the second week of parabiosis it became impossible to demonstrate a cross circulation, and the once-united dermal epithelium of the parabionts separated as the subjacent incompatibility stromal reaction developed. Despite this extent of reversal of healing, the ears of the parabionts remained firmly united by bundles of collagen and reticulum which were surprisingly resistant to the generally destructive effects of the incompatibility reaction.

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RECENT DEVELOPMENTS IN ENVIRONMENTAL CANCER

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(Continued from page 399)

B. Organic Chemicals

1. *Benzene.*—During the past 15 years numerous additions have been made to the list of cases showing that a prolonged, low-level occupational exposure to benzene may result in the development of leukemia. It seems to be well established that such contacts may elicit not only aplastic anemia and panmyelophthisis, but also hyperplastic reactions of the erythropoietic and leucopoietic tissues (agnogenic myeloid hyperplasia), evidenced in the peripheral blood by the appearance of erythrocytoses and hyperleucocytoses of sometimes leukemoid proportions.* More recent observations on benzene workers have shown that there may occur gradual transitions from such hyperplastic reactions into definitely leukemic conditions, they being either of the myeloid or lymphoid type or, occasionally, assuming the character of lymphosarcoma,† chloroleukemia,[295] or erythroleukemic myelosis.[296] Myeloid leukemias of an acute or chronic variety were reported in 19 cases,‡ and myeloid aleukemia and lymphoid leukemia were seen twice each.§ In one case acute leukemia followed upon exposure to naphthol.[315] Thus there is on record a total of 28 cases of cytologically identified malignant hematopoietic reactions following an exposure to benzene, which may be extracted from the bone marrow many months after cessation of exposure.

Some evidence exists indicating that the course of events seen in the development of benzene leukemia from transitory hypoplastic agranulocytic reactions followed by a passage through hyperplastic and leukemoid reactions into the full-blown leukemia is characteristic of the symptomatic pattern of leukemia in general.‖ The completion of the entire cycle may take several years. It seems, moreover, that myelotoxic chemicals which may elicit allergic agranulocytic episodes furnish the specific stimulus for such developments.¶

2. *Aromatic Amino and Azo Compounds.*—The last 10 years have seen many accessions to the list of publications on carcinogenic aromatic amines and related azo compounds. An increased interest in the carcinogenic action of these chemicals

National Cancer Institute, National Institutes of Health, Public Health Service, and Department of Health, Education and Welfare.

* References 208-214 and 278-292.

† References 280, 293, and 294.

‡ References 297-310.

§ References 311-314.

‖ References 208-214 and 230-233.

¶ References 216 and 234.

has developed not only because of the recent demonstration of occupational bladder cancer among workers in aniline dye plants of several countries in which they had not previously been discovered (France, Italy), but also on account of the continued, and in part increased, appearance of such tumors among aniline dye manufacturers of countries in which they had been reported before. The successful production of cancers in experimental animals by the administration of other aromatic amino and azo compounds, particularly dyes employed in the coloring of foodstuffs, cosmetics, and drugs brought about an active revival of interest in these compounds, which had lagged for many years following the initial discovery of bladder cancer among fuchsin producers.

Table 19 shows strikingly the march of the aromatic amine cancers of the bladder through the different countries some 10 to 20 years after the establishment of aniline dye manufactures.

TABLE 19.—*Time Table of the Discovery of Aromatic Amine Cancers of the Bladder in Various Countries*

Country	Discoverer	Year	Approximate Total Number of Papillomas and Carcinomas Up to 1950
Germany	Rehn	1895	350
Switzerland	Scheidler	1905	190
Great Britain	Ross	1918	225
Austria	Schüller	1932	2 (?)
Russia	Rosenbaum and Gottlieb	1936	75
United States	Ferguson and others	1934	250
Italy	di Malo	1936	90
Japan	Nagayo and Kinosita	1940	7 (?)
France	Billiard-Duchesne	1946	41

The available information, which, however, is not identical with the published records, notes the occurrence of approximately 1,500 cases of occupational bladder tumors reported from all sources. Since 1941 # the following additional contributions were published on the occurrence of aromatic amine cancers of the bladder: United States, none (last report in 1938 noting about 100 cases); Great Britain, 10 *; Germany, 3 †; Switzerland, 4 ‡; Italy, 4 §; France, 5 ||; Russia, 1 [344]; Japan, 1.[345] Recent observations by Case and co-workers [224] and Chute [346] suggest that an aromatic amine bladder cancer hazard may exist also for rubber workers exposed to antioxidants containing beta-naphthylamine as a contaminant.[350] It may be mentioned, moreover, that, according to Hamilton and Hardy,[347] a case of bladder cancer in a trinitrotoluene worker was accepted in Germany as compensable because the chemical is excreted in the urine as an amino compound. Williams,¶

References 208-214.

* References 316-325.

† References 326 and 327; Wolff: Personal communication to the author.

‡ References 328-331.

§ References 332-335.

|| References 339-343.

¶ Williams, M. H. C.: Personal communication to the author.

who produced cancer of the bladder in dogs given 4-amino diphenyl, suggested that the production and use of rubber antioxidants made from this amine might be associated with a similar hazard to man. English observations, moreover, indicate that bladder cancer hazards are connected with the manufacture of auramine and magenta.

It can be considered as established that at least two aromatic amines, namely beta-naphthylamine and benzidine produced for the manufacture of dyes and related products, possess carcinogenic properties and cause cancers of the urogenous organs, particularly the bladder. Of these two chemicals, beta-naphthylamine appears to be, according to plant experience, the more dangerous one. An occupational exposure to this substance for only six months seems to be sufficient to elicit bladder tumors in some individuals after a latent period of some 10 to 15 years. Excessive, uncontrolled exposure to these amines was in former decades associated with a bladder tumor attack rate approximating 100% of the worker population at risk. In Basle, bladder cancer was found to be 33 times as common among dye workers as among the males of this region.[351] English investigators place the risk of contracting tumors of the bladder at about 33 times that of the population at large for "mixed exposures" to aromatic amines, at 16 times for alpha-naphthylamine, 61 times for beta-naphthylamine, and 19 times for benzidine.[388]

The claim recently advanced by Rhoads [383] that beta-naphthylamine is almost without effect in the female is obviously incorrect, since the experimental production of bladder cancer with this chemical was first achieved exclusively on female dogs, not only by Hueper and co-workers,[327] but even by Rhoads and co-workers (Hartwell [403]). The additional suggestion of Rhoads to administer estrogens prophylactically to workers occupationally exposed to beta-naphthylamine for the prevention of bladder cancer is obviously of highly dubious merit.

A number of other aromatic amines were and, in part, still are suspected of possessing carcinogenic properties. Thus, *o*-toluidine and dimethylaniline are thought to have been the cause of bladder cancers observed in fuchsin producers during the early days of the industry. Aniline does not seem to be carcinogenic to man, although it was originally suspected to be so and, for this reason, has given its name to these occupational cancers ("aniline tumors"). Bladder tumors have also been observed among alpha-naphthylamine workers in this country and abroad. It is at present assumed that the presence of about 3 to 5% beta-naphthylamine in the alpha-naphthylamine is responsible for these neoplasms.

The carcinogenicity of beta-naphthylamine and benzidine has been confirmed in animal experiments (beta-naphthylamine, dogs [bladder cancer] # and mice [fibrosarcoma] *; benzidine, dogs [bladder cancer] and rats [hepatomas, ear duct carcinomas, leukemias] †). There is, moreover, evidence that apparently some of the urinary metabolites of these amines are also carcinogenic. Hueper ‡ produced, with an impure 2-amino-1-naphthol obtained by Wiley from the urine of dogs fed beta-naphthylamine, several intraperitoneal tumors after repeated intraperitoneal injec-

References 208-214, 348, and 352.
* References 353 and 354.
† References 352 and 355.
‡ References 208-214.

tions of an oily solution. Using pure 2-amino-1-naphthol incorporated into paraffin pellets and placed into the bladder lumen of mice, Bonser and co-workers § obtained papillomas and carcinomas. The subcutaneous introduction of this metabolite into mice also resulted in the development of cancers, while the intravesical administration of beta-naphthylamine into mice did not cause tumor formation. The feeding of this chemical to mice, dogs, rats, rabbits, and guinea pigs, on the other hand, by various investigators || was followed by the development of hepatomas. In recent studies of Bonser and co-workers on the excretion of 2-amino-1-naphthol after the administration of beta-naphthylamine to dogs, rabbits, rats, and mice, it was shown that there exists a parallelism between the relative amounts of 2-amino-1-naphthol excreted and the development of bladder tumors in the different species.

Similar claims as to the noncarcinogenic character of the original aromatic amine and the carcinogenic action of the hydroxy metabolite were advanced by Baker. There is, according to Baker, no evidence that benzidine base is carcinogenic. The subcutaneous injection of a metabolite of benzidine, namely 3,3′-dihydroxy-4,4′-diaminodiphenyl, into mice resulted in the development of tumors of the bladder and liver.¶

The high frequency of primary cancers in other organs in addition to those present in the urogenous system of dye workers suggests that these aromatic amines operate not only by way of the urine but also by hematogenous spread.#

There are a number of other aromatic amines which have produced tumors in experimental animals upon oral medication or parenteral introduction (4-aminodiphenyl; 3,2′-dimethyl-4-aminodiphenyl; N,N-dimethyl-4-aminodiphenyl, *p*-toluidine; *o*-toluidine; acetylaminofluorene, and others) * and which may occur as contaminants or intermediaries in certain chemical operations. Cancers have been elicited by these chemicals in various organs, including the bladder, liver, brain, intestine, etc., of rats and/or dogs.

Of special interest in connection with environmental carcinogenesis, however, is the demonstration of carcinogenic properties of some aniline dyes, since they are used as coloring agents for many industrial purposes, as well as in foodstuffs,† cosmetics, and drug preparations,‡ while others, such as Evans blue, toluidine blue, scarlet red, and fuchsin, are employed as medicinal agents,§ for fumigating purposes,[398] and as gasoline dyes.[399] While several investigators have suggested that the former and/or continued use of butter yellow (*p*-dimethyl-amino-azobenzene) as a food dye (butter, margarine, rice, etc.) has caused a rise in the liver cancer incidence in the Austrian population in recent years [389] or is responsible in part for the high incidence of this neoplasm among Oriental peoples,|| there is no reliable evidence available supporting such a contention.

§ References 356-358.
|| References 45, 352, 356-358, 362, 384, and 385.
¶ References 359-361.
# References 328, 363, and 364.
* References 366-368.
† References 400-403.
‡ References 370-379.
§ References 380-383.
|| References 400 and 401.

There is, on the other hand, abundant evidence on hand attesting that a prolonged dietary intake of this dye and related food dyes (oil orange yellow [benzene-azo-beta-naphthol], oil yellow H. A., and azo compounds) may elicit in rats, mice, and dogs placed on a vitamin B complex (riboflavin)-deficient diet, tumors of the liver and/or other organs (bladder, ear duct, etc.).¶ It was shown, moreover,[408] that there was a potentiated synergistic effect on hepatoma formation in rats when two different amino compounds or azo compounds were simultaneously administered.[408] The observations of Druckrey,# with chrysoin SGX (2,4-dioxyazobenzol-2'4-sodium disulfonate), show that only some of these dyes have carcinogenic properties. Mention may also be made of repeated experimental demonstrations of carcinogenic properties of several certified food dyes used in this country and abroad, belonging to the triphenyl methane group (light green SF, brilliant blue FSF, fast green FCF), when these dyes were repeatedly injected into the subcutaneous tissue of rats, where fibrosarcomas arose.* No tumors, however, developed when the dyes were given orally. Recent investigations with a specially purified neutral light green SF undertaken by Gross showed that the carcinogenic effect was present with the pure dye and thus was not the product of some impurity.† Brilliant blue FSF has been removed from the list of certified food dyes and light green SF from that of the cosmetic dyes permitted in Germany.

Whether or not the appearance of sarcomas of the lymphoid type in rats fed amaranth, ponceau SX, light green SF yellowish, Guinea green B, and hematoxylin is actually due to the dyes given needs confirmation.[389] Rhodamine B, a xanthine dye, when injected subcutaneously into rats produced fibrosarcoma, but was innocuous when given by mouth.[390] Reticulum cell sarcomas of the liver were obtained when trypan blue was repeatedly injected into rats.‡ Since congenital malformations were observed in the offspring of rats treated with trypan blue, there is a possibility that this dye may also have a transplacental carcinogenic effect,[393] similar to that exerted by urethan.

Finally, mention may be made of the production of fibrosarcomas in the subcutaneous tissue of mice following the injection of sulfonamides § and of renal adenocarcinomas, abdominal fibrosarcomas, and lymphosarcomas in rats and of round cell sarcomas in mice following the subcutaneous implantation of sulfonamide pellets.[396]

The evidence on hand indicates that aromatic amino and azo compounds possess carcinogenic properties to an extent which makes thorough and competent investigations of all such chemicals which have close contact with man an urgent necessity. It is apparent, moreover, that possible cancerous reactions from exposure to carcinogenic members of this large group of chemicals are not necessarily restricted to the organs of the urogenous tract, but may manifest themselves in other tissues and organs, such as the liver, hematopoietic tissues, and intestine.

3. *Coal Tar, Pitch, and Creosote Oil.*—Occupational contacts with coal tar, pitch, soot, and creosote oil has furnished during the past 12 years a considerable

¶ References 402-407.
# References 409 and 410.
* References 386-388.
† Druckrey: Personal communication to the author.
‡ References 391 and 392.
§ References 394 and 395.

number of new cases of cancers of the skin, at least in Great Britain, where, by now, for over 30 years epitheliomatous lesions caused by coal tar and mineral oil have been reportable with the Chief Inspector of Factories and Workshops.|| The stimulus provided by such a statutory notification upon a more general recognition of the environmental causation of epitheliomata is clearly discernible from a graph of Henry showing the number of notified cases during the period of voluntary notification (1911 to 1920, with 182 cases) and that of the statutory notification (1921 to 1949, with 6,624 cases) (Fig. 3). The annual figure of cases increased in England and Wales from 45 cases, in 1920, to 245, in 1946, dropping to 190 in 1949. A total of 25 skin cancers was attributable to contact with creosote oil among creosoters of wood and warehouse workers; 2,652 had exposures to coal tar and pitch, 1,658 to shale oil and mineral oil, and 312 had contact with both coal tar and oil.

The large number and variety of occupations which contributed these exposures are especially instructive to those whose professional activities are related to the technologic, sanitary, and medical control of the carcinogenic agents of our modern industrial environment. Equally profitable is a study of the occupations which, according to Henry, show an excessive incidence rate of cancer of the skin, penis, and scrotum. Additional official data on the number and sites of skin cancers attributable to occupational contacts with coal tar products and the occupations involved are contained in the annual reports of the Chief Inspector of Factories.¶

British investigators have furnished other excellent epidemiologic studies on occupational coal tar and pitch cancer, dealing with incidence rates among special occupational groups, age distribution, length of exposure time and of latent period, influence of complexion on susceptibility, preventive measures, therapeutic procedures, and similar aspects.# Irwin and Goodman [425] contributed statistical methods of measurements of carcinogenic properties of tar, while Berenblum and Schoental * studied its carcinogenic constituents. The various coal tars, arranged in ascending order of the degree of cracking they have undergone, and thus the amounts of naphthalenes, anthracenes, and similar complex ring compounds they contain, appear as follows: (1) low temperature tar, (2) blast furnace tar, (3) vertical retort tar, (4) horizontal retort tar, and (5) coke oven tar.[418] This order parallels roughly the scale of carcinogenic potency of the various tars. Since the treatment of skin diseases with coal tar preparations is still much in vogue,† it is of interest that Berenblum produced cutaneous cancers in mice painted with solution of coal tar (B. P.).[430]

The original observations of Kawahata ‡ on the excessive incidence of lung cancer among generator gas retort workers in a Japanese steel mill (5 per 1,000) have recently been confirmed by similar findings among English and Canadian gas producers, i. e., mainly retort workers and stokers § who inhale tar fumes. It

|| References 411-413.
¶ References 237 and 414-416.
# References 417-424.
* References 426 and 427.
† References 428 and 429.
‡ References 431 and 432.
§ References 433 and 434.

CUTANEOUS CANCER IN RELATION TO OCCUPATION

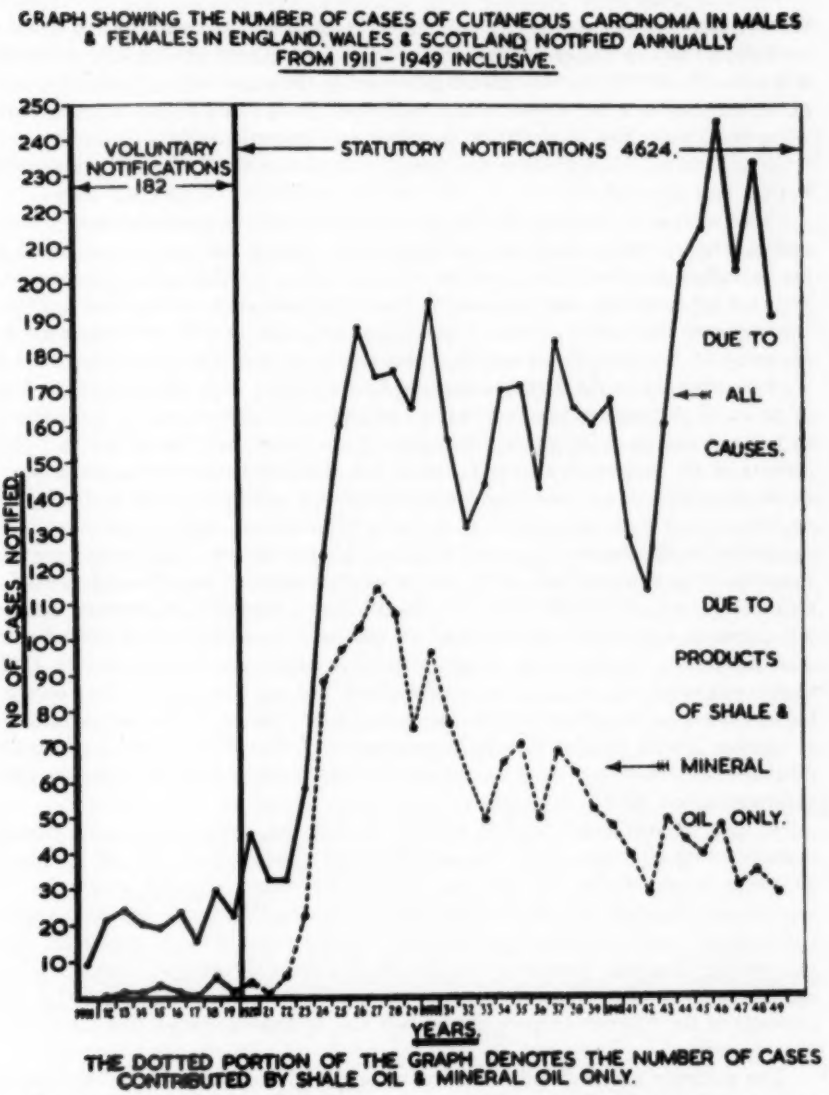

Fig. 3.—Cutaneous cancer in relation to occupation. Graph showing the number of cases of cutaneous carcinoma in males and females in England, Wales, and Scotland; notified annually from 1911 to 1949 inclusive.

appears, therefore, that a frequent occupational inhalation of tar fumes produced by the coking of coal conveys an excessive liability to cancer of the lung. In a recent analysis of the occupational diseases among workmen of Swiss gas coking plants, Menz [435] stated that 21 of 93 deaths (22.6%) were caused by cancer.

Reports from other countries show that there were notified with the Chief of the Medical Inspection, Ministry of Labor in Belgium, between 1933 to 1947, a total of 22 cases of cancer of the skin due to an occupational exposure to tar, pitch, and mineral oil.[436] There was placed on record in Germany only one case of cancer of the scrotum in a tar worker and a cancer of the skin in a carbon-black worker, while none were seen in electrode manufacturing operations.[437]

From France a single acute traumatic tar cancer of the lip burnt by a drop of hot tar was reported.[438]

Somewhat less scanty are the reports on occupational tar cancer from the United States. The occurrence of 5 cases of skin cancer among 100 workers employed in the manufacture of electric conduits covered with pitch-impregnated paper was reported by Schwartz and Tulipan.[439] Two additional cases in coal tar distillery workers and the first American case of creosote skin cancer were recorded by Downing.[440] Information is available, moreover, on at least nine skin cancers among workers engaged in the impregnation of paper conduits with molten pitch and on 25 cases of skin cancer observed within an eight-year period among the workers of a tar distillery. Subsequent investigations, moreover, established the fact that cancers of the lung were also seen among tar distillery workers who are exposed to the inhalation of tar fumes during the distillation process, as well as during the manufacture of various products made from tar fractions, such as tar paint used especially for the coating of gas and oil pipes. There can be no doubt that the great majority of occupational tar, pitch, and creosote cancers of the skin and, possibly, of the lung which occurred in this country have remained unreported and/or unrecognized, considering the fact that the number of workers having contact with such products is considerable. In the Pittsburgh area alone a total of over 4,000 workers is employed in by-product coke plants and gas generators. That occupational exposures to anthracene oils also exist in the United States is indicated by a warning issued by the Illinois Department of Labor [441] to guard against the inhalation of anthracene oil fumes produced when roofing pitch is heated to temperatures above 400 F.

It may be mentioned in this connection that the newly developed industry manufacturing oils, tars, and waxes by the direct (Bergius) or indirect (Fischer-Tropsch) hydrogenation of coal may also be associated with the generation of carcinogenic material. Recent investigations of Hueper [442] on experimental animals with a larger number of these products showed that some of them, particularly the high-boiling fractions, possess a considerable degree of carcinogenic potency when applied to the skin or injected intramuscularly. There is some suggestion that the products of the Fischer-Tropsch gasification and hydrogenation process may cause liver tumors.

The evidence on hand indicates that occupational exposures to coal tar, pitch, creosote oil, anthracene oil, and the asphalts and carbon blacks made from coal combustion products are associated with distinct cancer hazards to the skin and respiratory organs. It is as yet uncertain whether such hazards extend to other organs, such as the hematopoietic tissues and the bladder, although a few scattered

observations suggest such effects as to the production of leukemia, while English statistical evidence shows a positive correlation of gas workers to cancer of the bladder.‖

Since the question of a carcinogenic action of wood tar has repeatedly been raised in recent years,¶ it may be noted that Henry # is uncertain whether several skin cancers notified were attributable to wood tar. Wood soot obtained from the chimney of a smoked sausage factory, when applied to the skin of rats or implanted subcutaneously, elicited cancers in a moderate number of animals.[445] However, tumors were not obtained when the soot was given orally. Since skin cancers have been observed among turpentine makers, the problem needs further investigation because of the fact that exposures to wood soot exist for occupational, dietary, medicinal, and general environmental reasons.

4. *Shale Oil and Lignite Oil.*—No new cases of cancer of the skin or lung caused by exposure to tar and oils obtained by the retorting of lignite have been reported in recent years from Germany. It is possible that most of these carbonaceous resources have been used for the production of oils and related products by the Bergius and Fischer-Tropsch procedures. Cancers of the skin have not been observed so far among the German workers employed in these manufactures, according to information received from competent German observers.

Exposure to shale oils, i. e., crude paraffin oils and lubricating oils, by manufacturers and consumers of these oils has continued to give rise to cancer of the skin in Great Britain. Mule spinners' cancer affecting mainly the scrotum, vulva, and forearm has remained an active source of occupational cancers in England and Wales,* despite great efforts made toward the production of a noncarcinogenic lubricating oil † and the development of mechanical devices reducing the splashing of lubricating oil from the rotating spindles. Special mention is deserved here of the rather recent appearance of cancer of the vulva among female workers employed in cotton-spinning operations.‡ Through a special survey made, Henry ascertained that over 59% of the women with vulvar cancer observed in six hospitals had been cotton operatives, although only 28% of the admissions were of women related to the staple cotton industry. The occurrence of skin cancers among brick manufacturers coming in contact with the mineral oil used for coating the molds was emphasized by Keatings and Potter.[451]

Studies on the carcinogenic constituents of shale oil and related aspects were published by Berenblum and Schoental.§ The presence of 3,4-benzpyrene in blue shale oil was demonstrated through characteristic fluorescent spectra.

Recent bioassays made of American shale oil produced in a pilot operation by the Bureau of Mines showed that American shale oils boiling above 600 F. also possess carcinogenic properties, although the samples tested were less potent than corresponding Scottish oils.‖ It seems appropriate to keep such observations in

‖ References 411-413.
¶ References 443 and 444.
# References 411-413.
* References 411-413 and 448-450.
† References 446 and 447.
‡ References 411-413.
§ References 452 and 453.
‖ References 442 and 454.

mind when using shale oil preparations in the prolonged treatment of dermatologic conditions ¶ if medicinal cancerous sequelae some 10 to 30 years later are to be avoided.

The inhalation of shale oil droplets suspended in the air of spinning rooms have, according to Kennaway, not produced an increased liability to lung cancer among the spinners. While Zeglio [457] reported the presence of respiratory symptoms among manufacturers and users of tars obtained from bitumen, there is no reference made to lung cancer.

Although shale oils are produced in many countries (Scotland, France, Germany, Esthonia, Australia, Sweden, and United States), occupational cancers caused by these agents have so far been reported from Great Britain only. Since the manufacture of shale oils in the United States is of rather recent date and still in an experimental stage, occupational cancer hazards are here mainly problems of future developments.

5. *Petroleum and Petroleum Derivatives.*—There have been few published reports on the occurrence of occupational cancers of any organ because of contact with petroleum products from any country during the past 12 years. Petroleum products of high boiling point are extensively used in industry for a great variety of purposes, such as industrial, domestic, and diesel fuels; lubricating, cooling, cutting, and quenching oils; road asphalts and tars; rubber plasticizers; paints; raw material for carbon-black production; insulating material, and building materials.

From American sources there are on record a few general statements on the carcinogenicity of certain petroleum products observed among producers and consumers, without any definite numerical and occupational data.# Schwartz, Tulipan and Peck [461] reported that among 743 oil field workers in California, 146 had keratotic lesions on hands, forearms, face, and neck, of which 5 were epitheliomatous and found among 209 well pullers heavily exposed to crude petroleum (2.5%). They had been exposed to the oil for from 9 to 30 years and were mostly of fair complexion. A study of 330 oil field workers in Texas, on the other hand, did not show any abnormal incidence of epitheliomas. It may be possible that the difference in behavior in the skin cancer incidence between oil field workers in California and those in Texas is due to different carcinogenic properties of the crudes, since some such differences have, at least experimentally, recently been found.[462] On the other hand, consideration must be given to the fact that workers in oil fields in both states become exposed to intense sunshine. Since it cannot readily be assumed that Texas sunshine is less potent carcinogenically than California sunshine, there remains some doubt as to the actual significance of these observations.

Of definite importance, however, is the recent rediscovery of the occurrence of scrotal cancer among American oil refinery workers employed in the paraffin pressing operation.[463] According to Peller, citing from a communication of R. E. Eckardt and N. V. Hendricks, there were eight scrotal cancers among a total of 83 cancers of all sites among a refinery population of 2,500 during 1937 to 1948. All of the scrotal cancers plus seven internal cancers occurred in the small group of about 120 pressmen of the wax and paraffin department (scrotal cancer rate among pressmen 312 per 100,000, as against 0.15 among the male population at

¶ References 455 and 456.

References 458-460.

large per year). It may also be of significance that five of the nine cancers of the lung observed among employees of the refinery were present in workers of the paraffin department, where an exposure to oil fog exists. It is evident from these observations that some American petroleum products occurring in refineries are carcinogenic and that apparently small groups of workers are mainly exposed and thus show an excessive liability to skin and lung cancer. Incidence rates for cancer among oil refinery workers, therefore, are likely to give incorrect results as to the existence of carcinogenic oils if they are calculated for the entire complement of a refinery with disregard of these pertinent facts.[464]

Perhaps mention may be made in this connection of the fact that in an oil refinery a considerable number of things are handled which have recognized or suspected carcinogenic properties (asbestos, smoke, welding fumes, benzene, chlorinated hydrocarbons, radioactive materials, phenyl beta-naphthylamine, chromium, coal tar, bitumastic, pitch, nickel salts, petroleum oils of various types, untreated wax, and ultraviolet radiation).[465] While it is not likely that all these agents come in contact with all refinery workers all of the time, there is a certain probability that some of them provide effective exposures to some of the workers for some of the time, if not all of the time.

A petroleum refinery with its petrochemical attachment, which in the types and variety of environmental chemical agents handled resembles in many respects a rubber-manufacturing establishment, thus appears to be a place where contacts with carcinogenic chemicals should not infrequently occur if no special precautionary measures are taken.

Equally scanty as to the number of petroleum cancers notified are the records of the Chief Inspector of Factories in England and Wales. Henry suggested that perhaps such cases were not reported and that some steps might have to be taken to improve this situation. In a survey of workers exposed to cutting oils by skin contact and inhalation in automatic machine shops, Cruickshank and Squire[466] found that there occurred with lengthening employment an increase in the number of warts and hyperkeratoses located on the hands and forearms as well as, less often, on thighs or knees. An analysis of the cases of scrotal cancer seen in the Birmingham area revealed that in 12 out of 34 cases an occupational exposure to cutting (six), lubricating, or quenching oils had been present. Only one of the 12 had worked outside the engineering industry. A sample of used cutting oil which was tested on mice (C57) by skin application produced merely local irritation, while rabbits developed papillomas. Since cutting oils are often complex mixtures of various ingredients, including petroleum oils or shale oils, it remains open whether or not the observations of Cruickshank and Squire incriminate fully the petroleum fraction contained in the cutting oils as the carcinogenic factor. It is apparent, however, from a report of Auld[467] that the English petroleum industry is alerted to the possibility of industrial cancer hazards related to the production and use of various petroleum products.

The singular observation of the appearance of papillomas of the scalp, treated for 10 years with Brilliantine, containing mineral oils, and subsequently of papillomas on other, nonexposed parts of the body, all of which disappeared within four weeks after the applications were discontinued, is of very doubtful significance as to a potential carcinogenic action of petroleum products, especially as the possibility exists that such preparations may have contained coal tar derivatives.[468]

Increased attention has been given in recent years to the possibility that the inhalation of mineral oil mist or fumes from high speed machinery, from quenching baths in foundries, from high pressure grease guns and diesel jets, from spraying of metal parts with oil, and from high temperature operations (refineries, core making, and other foundry processes), or the aspiration of medicinal mineral sprays, may result in the production of lung cancer.

Observations of several French investigators lend some support to this concept.* Huguenin and co-workers found that among 112 cases of lung cancer, 18 were metallurgical workers, 8 were chauffeurs, 5 mechanics, and 1 an engineer (28.5% of the group exposed to inhaled oil). In view of the fact that there was observed, as mentioned above, a highly excessive lung cancer incidence among paraffin pressers who were exposed to an oil of established carcinogenicity, and considering the isolated occurrence of lung cancer in persons with liquid petrolatum (lipid) pneumonia,† the likelihood of the actual existence of causal relations between the inhalation of finely dispersed mineral oil and lung cancer is great indeed.

Extensive experimental studies conducted during recent years, mainly under the sponsorship of the petroleum industry in the United States and England, have clearly established the fact that an appreciable number of high boiling oils (fuel oils and lubricating oils) possess carcinogenic properties for the skin of mice, rabbits, and monkeys.‡ Since most of the various fractions studied are products of the catalytic cracking process introduced about 10 years ago, no human cases have as yet come to observation. There is, however, no doubt that cancer hazards from such oils are not restricted to employees of the refineries, but extend to the consumers of these products when they are used in a nonmodified form. There is, moreover, some experimental evidence indicating that carcinogenic contaminants are present in some of the lower boiling fractions used as vehicles and basic chemicals.

Experimental investigations of recent years have furnished some observations on the occurrence of intestinal neoplasms in mice and rats after the ingestion of various types of mineral oil.§ These findings are of some potential human importance, since mineral oils are used for medicinal purposes, have been employed as substitutes for vegetable oils in salad dressings, as shortening in baked goods, and as pan greases. The last-mentioned use is particularly objectionable, because the mineral oil constituents of such greases are repeatedly heated to relatively high temperatures and thus originally noncarcinogenic products may be converted into carcinogenic ones.‖ It has been shown, moreover, that emulsified mineral oil penetrates the intestinal mucosa and accumulates in the mesenteric lymph nodes,[484] thus creating long-term exposures.

Finally, mention may be made of the accidental introduction of fuel and lubricating oils under high pressure into the human tissues (diesel jet and grease gun injuries).¶ Malignant sequelae from such accidents have not been reported.

* References 469 and 470.
† References 471 and 472.
‡ References 473-480.
§ References 474 and 481.
‖ References 482 and 483.
¶ References 485-487.

Serious attention must be given in the future to the possibility that cutaneous or respiratory contact with the sooty, incomplete combustion products of carcinogenic petroleum oils (carbon blacks and soot) may cause cancer of the skin or lung. It has been established that carbon blacks contain carcinogenic hydrocarbons and that benzene extracts of carbon blacks when applied to the skin of mice produce cancers.# The apparently normal cancer incidence among carbon black manufacturers reported by Ingalls [491] is based on data which readily can give a distorted picture, because the great majority of the workers included in the analysis had been employed for too short a period of time to have passed even the minimum length of latent period, while no attempt was made to ascertain the fate of former workers, especially deceased ones. The problem of cancer hazards among producers and users of carbon blacks (carbon black industry, rubber industry, ink industry, printing industry, paint and lacquer industry) is in need of a thorough and competent reinvestigation.

6. *Isopropyl Oil.*—The most recently discovered occupational cancer is represented by the cancers of the respiratory system (paranasal sinuses, larynx, and lung) in isopropanol manufacturers.[492] The first case appeared in 1937, about 10 years after the establishment of the manufacture of isopropyl alcohol from propylene gas. So far a total of 12 cases has been observed among isopropanol workers of two different companies. The majority of these tumors were found in the nasal sinuses (seven); the remainder involved the larynx (four) and the lung (one). The attack rate is high, considering the fact that 7 cases were found among a total of 71 workers employed in this operation for more than 5 years and among 31 employed for more than 10 years; i. e., cancers of the respiratory tract developed in 8.4% of employees exposed for more than 5 years. It was calculated that in one plant the incidence of sinus and larynx cancer per year per 100,000 was 134.5, as against an expected incidence of 6.3. While the absolute numbers of these new occupational cancers so far observed are still relatively small, their recognition repeats a pattern of discovery several times found in the past (bladder cancer in fuchsin workers, lung cancer in chromate workers, etc.).

The actual nature of the causal agent is still uncertain. It appears from the evidence that it might be contained in the somewhat volatile crude liquor (isopropyl oil) from which the isopropanol is distilled. There is no evidence that it is isopropanol, but possibly it is one of the by-products, such as isopropyl ether, isopropyl peroxide or epoxide, mesitylene, or polypropylene. It may be of significance that some of these agents have a tendency toward polymerization and that low-polymer condensates formed while the original material is in contact with the tissues are the actual causal agents. This concept is advanced in view of the recent demonstration of carcinogenic properties exerted by a considerable number of synthetic polymers (polyethylene, polyvinyl chloride, polyamides, Cellophane, Bakelite, etc.) and the formation of "polymer cancers" in rats and mice following their parenteral introduction.

Upon observations made during a personal study of the exposure conditions prevailing in one of the plants involved, on which proposals of the subsequently executed experimental work were based, it became apparent that cancers seemed to occur especially often among foremen who at times of accidents, such as breaks

References 488-490.

in pipe lines, leaking pumps, and similar defects causing leaks of the isopropyl oil, sustained a severe, and sometimes prolonged, exposure to isopropyl oil vapors. These findings demonstrate again the fact that workers engaged in maintenance and repair work providing opportunities for transitory intense exposures to industrial carcinogenic materials have a special liability.

In the absence of conclusive experimental evidence as to the nature of the causal agent, it remains uncertain whether the cancers in isopropanol manufacturers represent manifestations of cancers caused by aliphatic chemicals, cyclic compounds, or polymerized condensates. Since isopropanol is used not only as a purified product for medicinal purposes, but also in huge quantities as a technical product in industry

TABLE 20.—*Number of Skin Cancers per 100,000 Population in Six Urban Areas*

Region	City	Rate	Percentage Rate for White Males of All Cancers *
Northern area	Detroit	24	12.3
	Chicago	25	12.5
	Pittsburgh	37	16.0
Southern area	New Orleans	129	26.0
	Dallas; Fort Worth	110	....
	Atlanta	157	38.5
	Birmingham	..	42.3

* These rates parallel rather closely the percentages of total possible sunlight for the different communities or regions (Dallas, Texas, 60 to 80%; New Orleans, 62 to 64%; Pittsburgh, 50 to 57%; Detroit, 40 to 45%). Peller,[496] quoting from work of H. F. Dorn, presents the following data on skin cancer incidence per 100,000 population in three parts of the country:

Region of Cities	Incidence of Skin Cancer	
	Males	Females
North	22	18
South	116	70
West	41	34
Whites	38	28
Negroes	5	4

(rubber, paint, etc.), further inquiries on the possible presence of respiratory cancer hazards among employees of such operations are in order.

C. Physical Agents

1. *Ultraviolet Radiation.*—Recent years have brought a great number of epidemiologic studies originating in several countries which attest the causal connection between exposures to solar radiation and the development of cancers of the exposed skin.* McDowell,[493] giving the prevalence rates of skin cancer in different parts of the United States, presents the listing in Table 20.

Similar observations on the direct relation between exposure to solar radiation and cancer of the exposed skin have been made in Argentina, Colombia, Australia, Kenya, and South Africa. It is uniformly reported that fair-complexioned persons are much more susceptible than brunettes, and that nonwhites show a decreasing susceptibility with increasing skin pigmentation. While it is usually stated that Negroes are refractory to solar cancer of the skin, this may not be entirely correct.

* References 123, 128, and 493-508.

It is not unlikely that the normal resistance provided by their normally high pigmentation is absent during the reepithelization of the frequently observed ulcers of the lower extremity, and that this factor explains at least in part the relatively high incidence of skin cancer of the lower extremities of African Negroes, where these parts of the body are not covered by clothes and are thus exposed to sunlight. While it is doubtlessly correct that the degree of pigmentation is an important factor in determining the susceptibility of the skin to the carcinogenic action of ultraviolet radiation, recent experiments of Hueper [510] on haired and congenitally hairless rats, having a thick, cornified, corrugated skin have shown that the action of ultraviolet rays on the epidermis depends also upon the thickness of the covering cornified layer, because the hairless rats proved to be resistant to the carcinogenic action of ultraviolet radiation generated by a high-power mercury arc lamp.

Apart from environmental and occupational exposures to ultraviolet radiation contained in solar light, there occur also occupational exposures to artificial sources of this type of radiation.[512] The greatest interest has been attracted by exposures to ultraviolet rays contained in the electric welding arc.[513] While most of the reports on this matter deal with effects upon the eye, reference has repeatedly been made to the occurrence of cutaneous changes of the actinic type (chronic radiodermatitis) in arc welders.† Precancerous lesions on the lower lip of arc welders associated with and resulting from actinic dermatitis have been described.[516] Consideration must be given to the fact that effective exposures apparently can result from contact with refracted rays emerging from the lower edge of spectacle lenses and goggles,‡ giving rise to cancers of the face.

It has been stated that the active spectrum of the tumor-producing wave length is between 2,537 and 3,200 A.§ Recent experimental studies of Heller [522] tend to show that cancerous effects can be elicited in the skin of white mice by rays located on both sides of this range, especially if the exposures are combined with the simultaneous application of a photochemically active hydrocarbon, such as anthracene, i. e., when radiation with noncarcinogenic rays is combined with a sensitization by noncarcinogenic hydrocarbons. These observations, however, are not in agreement with those made by Rusch, Kline, and Baumann,[523] who found that the combined application of carcinogenic hydrocarbons and ultraviolet radiation does not exert any additive carcinogenic effects. So far, attempts at isolating from the radiated skin an extractable carcinogen have failed.[524]

From the information available, it appears that excessive exposure to sunlight accounts for the majority of skin cancers of the exposed skin seen in whites, especially those living in dry and sunny climates, at high altitudes, or occupationally exposed to a large amount of solar rays reflected from surfaces of water (sailors and fishermen).

2. *Thermic Radiation.*—The cancers not infrequently developing in scars and ulcers resulting from acute thermic trauma probably belong among the cancers caused by tarry substances produced by the carbonization of carbonaceous matter, such as tissues. This concept is supported by the fact that, so far, the so-called burn cancers have appeared only with third-degree burns.

† References 515-518.
‡ References 517 and 518.
§ References 502 and 519-521.

The existence of cancers of the skin caused by prolonged exposures to thermic radiation is less well established, since in most cases cited there existed also occupational contacts with soot, such as in railroad engineers and firemen or rollers in steel mills.[525] Henry || cited radiant energy as a cause of cancer of the skin for puddlers in the heavy metal industry; glass blowers and finishers; coopers; wheelwrights; hat plankers, formers, and stiffeners, and others. He was, however, not certain as to the exact kind of radiant energy active in such cases. Mattenci [526] contended from an analysis of 93 cases of skin cancer that basal cell cancers are elicited by infrared rays, while squamous cell cancers are due to ultraviolet rays. For this assertion, there is neither clinical nor experimental support. Both types of cancers are seen in man and rats after exposures to ultraviolet radiation.

3. *Ionizing Radiation (Roentgen Radiation, Radiation of Radioactive Substances).*—(*a*) Sources of and Population Groups Exposed to Radiation: The recent development of atomic energy and the production of numerous radioactive isotopes has greatly increased and spread the interest in environmental and occupational carcinogenic hazards from exposure to various forms of ionizing radiation (cosmic radiation; natural and synthetic radioactive substances [alpha, beta, and gamma radiation]; radiation generated by cyclotrons, synchotrons, betatrons, and similar accelerator devices [neutron radiation], and roentgen radiation). It has been suggested that cosmic radiation may exert a direct or contributory carcinogenic effect.¶ However, there is, with the exception of some contradictory observations on experimental animals, no valid epidemiologic evidence available which would support such a concept. In fact, populations living at sea level in the United States (Eastern Seaboard and California), and thus receiving a somewhat lower amount of cosmic radiation than those residing at high altitudes in the Rocky Mountain region have higher cancer mortality rates than the populations of the Rocky Mountain states.

The number of persons coming in contact with other forms of ionizing radiation has markedly risen during the past 12 years. The more general use of various devices producing x-rays, such as x-ray apparatus employed for medical, dental, industrial, and commercial (cosmetic and shoe store) purposes, x-ray diffraction apparatus, electron microscopes, television tubes, cathode tubes, and the greatly increased penetrating power and energy of the radiation generated from some of these sources, not only brought many more persons in contact with x-rays but also has accentuated the chances of incidental environmental or occupational total body irradiation by secondary scatter rays.#

It has been estimated that in the United States more than 125,000 x-ray units are being used for diagnosis (fluoroscopy, photography, and therapy), entailing a potential exposure to radiation of more than 215,000 medical and technical personnel engaged in their operation,[530] exclusive of the number of patients subjected to radiation of varying types and intensity. Some 15,000,000 persons were given chest x-rays in surveys in 1950, while many others were exposed during mass dental surveys.[531]

There are, in addition, approximately 2,000 industrial roentgenographic installations operated by some 5,000 persons. These units (roentgenographic and fluoro-

|| References 411-413.

¶ References 527 and 528.

References 529 and 530.

scopic) are used for the determination of defects in castings, fabricated structures, pressure vessels, and welds; for the detection of foreign material in packaged foods and "soft" goods, and for protection against theft and sabotage. It has been noted that portable units and fluoroscope inspection units on conveyor belts are the most probable sources of radiation exposure in industry. The use of high voltage x-ray equipment (1,000,000 volts and above) has increased the hazards from powerful scatter rays if the equipment is only partially enclosed or inadequately shielded.

The use of some 10,000 fluoroscopes for shoe fitting is associated with radiation exposures to some 30,000 to 40,000 operators and sales persons, in addition to those received by an unknown number of shoe store customers.* The use of cyclotrons, linear accelerators, and positive ion tubes in research laboratories has created additional opportunities for exposure to ionizing radiation. More than 100 particle accelerator units are in use.[530] Similar hazards are associated with the operation of x-ray diffraction units.[547] Several cases of skin ulcers resulting from accidental overexposures in the use of these units have been reported.†

Recent investigations have established the existence of a number of stray radiation sources (television picture tubes, radar cathode ray tubes, industrial cathode-ray oscillographs, and electron microscopes),‡ which may deliver low-level x-radiation to the operators of these devices. Although as yet the operating of such apparatus has been carried on by thousands of persons for periods of several years without known ill effects, this observation is no guarantee against the appearance some years hence of untoward reactions, especially of the hematopoietic system, from some of these devices whenever high intensity cathode tubes, such as television projection tubes and powerful "electron guns," are used.

The more extensive and diversified employment of radioactive substances and the large-scale production of artificially radioactive material for military, industrial, medicinal, and research purposes have furnished in recent years the main additions to the previously known sources of environmental, occupational, and medicinal radiation hazards. Potential exposure to ionizing radiation exists not only for the miners and millers of radioactive ores and the personnel of the atomic energy plants,[561] but also for persons present at experimental nuclear detonations and residents of areas in which radioactive sewage and wastes are released and where radioactive dust generated by smelters and atomic explosions may settle and contaminate the air, the soil, and, especially, the water supplies.§ Radioactive hazards exist also for the users of radioactive isotopes for medicinal purposes, for research, and for industrial operations, equipment, and products (luminous paint, static eliminators,‖ spark plugs, radium-radiographic units, vacuum gauges, gas mantles, thickness gauges, liquid-level gauges, well logging, interface markers in oil pipe lines, tracers in metal products, detergents, chemicals, pharmaceuticals, electronic tubes, and cold sterilization of food and drugs).¶

* References 530, 533, and 535-542.
† References 530 and 532-534.
‡ References 543-546.
§ References 529, 530, 533, and 548-552.
‖ References 530 and 553-559.
¶ References 533, 560, and 562.

The United States has become, during the last decade, the second largest producer of uranium ores. This industry now employs several thousand workers in mining and milling operations,[530] apart from the considerable number of persons engaged in the operation of atomic energy installations.

During the last war, many workers were employed in the production and application of radioactive luminous paints for marking instrument boards of airplanes, submarines, guns, and similar military equipment. After the war, a smaller number of workers, often not well supervised, became exposed to radioactive material while salvaging radioactive paint from this material.[529] Moeller and co-workers [530] remarked, in regard to the extent of radiation exposure in the United States, that radium and many other naturally radioactive materials may be purchased on the open market, that no formal application or special facilities are required in order to obtain these materials, and that numerous instances of radium being lost have been reported, all constituting danger of unsuspected radiation exposure.[563]

From an analysis of the reported data on the actual production of radiation injury from the various sources mentioned, it is evident that the existing radiation hazards are not always adequately controlled. The investigations of Harris [531] on personnel employed in conducting mass dental surveys on children indicated that a possible radiation hazard may exist, especially for the operating dentists, from exposure to x-rays scattered from the heads of the patients.

Radiation dermatitis, mainly affecting the hands, among radiologists and, especially also, among physicians, is still relatively common.# It is reported that an average of three cases of radiodermatitis in physicians are still being seen at the Mayo Clinic a week.* It appears that between 26 to 28% of all cases of chronic radiodermatitis proceed to become cancerous.† The introduction of radioactive isotopes into medical practice doubtlessly has introduced additional potential radiation hazards for physicians, nurses, cleaning personnel, laboratory technicians, chemists, physicists, and pathologists,‡ in addition to creating potential hazards to the patients treated § and to persons having contact with radioactive hospital wastes.

There can be little doubt that radiation injury in patients from the medical application of ionizing radiation is likewise still rather frequent. While such occurrences are justified and unavoidable when the actinic agents are applied in the diagnosis and treatment of diseases which endanger life and cannot be adequately controlled by other medicinal means, the observation of such complications in patients exposed to excessive ionizing radiation for more harmless conditions definitely requires special justification.‖ Recent investigations of Sulzberger, Baer, and Borota ¶ demonstrated that the use of superficial x-ray therapy for various nonmalignant dermatologic conditions, when competently applied, leads to rather few, and usually not serious, radiation sequelae. The significance of their observations as to the liability to late cancerous developments in the irradiated skin, however, is somewhat weakened by the fact that there were more cutaneous cancers

References 564-568 and 587.
* References 576 and 577.
† References 565, and 569, and 570.
‡ References 572 and 573.
§ References 562, 574, and 575.
‖ References 580 and 581.
¶ References 578 and 579.

among the members of their nonirradiated, control group than among those composing their test group. It can scarcely be maintained that any type of irradiation by x-rays exerts a protective action against the subsequent development of skin cancer.

The commercial and industrial use of ionizing radiation also appears to be ill controlled. All of the various reports cited on the use of x-ray apparatus for the fitting of shoes stated that the customer, as well as the sales clerks, run a potential risk of excessive exposures. In appreciation of these hazards, the sanitary codes of several states (New York and District of Columbia) have either regulated or prohibited their use. Various reports, cited above, have pointed out that definite radiation hazards have been connected with the improper handling, maintenance, and storage of polonium or radium-containing electrostatic eliminators, due to contact with gamma rays given off by radium or the inhalation of radon if the seal is broken; or by the inhalation, ingestion, and absorption of polonium liberated through breaking or flaking of the gold seal, or, possibly, under certain circumstances, of volatilized polonium.[530] Recent surveys of the exposure conditions existing for industrially used radiation equipment have also brought out the fact that the protective measures observed, as well as the medical supervision of the exposed personnel, are not always adequate.# Drinker [568] noted that "the use of x-ray equipment in the hospital of one Maritime Commission Yard forms an unwholesome chapter in the history of our shipyards," while Bourne and Cordier [569] found that only 2 of 42 plants in Ohio surveyed which use 60 roentgenographic and fluoroscopic units were able to produce a written report of a radiation survey.

The control measures instituted by industrial users of x-ray equipment have not kept pace with our knowledge of the harmful effects of radiation, which often become apparent only years or decades following contact with it. Mention may finally be made of a statement by Moeller and co-workers [530] concerning the observation of health damage from radioactivity among over 1,100 workers employed in the uranium mining and milling industry of the Colorado Plateau and subjected to a detailed medical examination by members of the Public Health Service. Such observations are scarcely surprising, considering the degree of exposure to radioactive dust, fumes, and gases which these workers sustained, and which surpassed, in some instances, many times the maximum permissible level.[571] Studies of gas mantle manufacturing plants, using thorium containing radiothorium as an impurity, indicated that concentrations of thoron in air may reach 400 times the maximum permissible level.[529]

Untoward reactions from exposure to ionizing radiation among employees of atomic energy establishments have remained, according to published reports, within the domain of occasional accidental occurrences.* It is maintained that the potential risks from excessive exposures for the employees of Atomic Energy Commission installations and for the residents living within the waste disposal zone of such enterprises are kept within the permissible limits. Since, however, the safety levels maintained during different periods of the operation of these plants have varied, it may be desirable to withhold final judgment as to the total elimination of health hazards, especially those of delayed occurrence, from these operations for an addi-

References 567-570.
* References 580 and 581.

tional decade or two. Because the latent period of such possible sequelae is much longer than the period which has elapsed since the present operations were started, and inasmuch as no data have been published on the state of health of large and competently analyzed groups of present and former long-term employees, it seems to be wise to discount as sources of conclusive information some of the publications on the nonexistence of occupational cancer hazards in these operations.†

The recognized potential and actual public health hazards associated with the production, handling, and disposal of radioactive material have formed the basis of regulatory and licensing legislation in various countries.‡ Dunlap § proposed, in view of these hazards, that "some legal restrictions might properly be established to limit the use of roentgen rays and radium to persons familiar with their potentialities for good and for evil."

The practicability of issuing comprehensive and definite regulations on such matters, however, is somewhat impaired by the fact that some uncertainty still remains as to the maximal permissible doses of x-rays and rays from the various naturally and artificially radioactive substances, and that this lack of definite information on pertinent data is even more marked in regard to the single or repeated individual dose of ionizing radiation which may produce cancers.[582] The maximum permissible exposure to the whole body or gonads at present recommended by the International Commission of Radiological Protection is 0.3 r per week measured in free air or 0.5 r measured on the body surface. In the case of hands and forearms, they recommend 1.5 r per week. Both these doses refer to externally originating x-rays or gamma rays of quantum energy less than 3 mev.[583] As little as 0.8 r per day may cause skin changes, while some changes of the blood may result from weekly exposures of 0.1 r.‖ At fluoroscopic work in hospital laboratories, exposures to more than 0.1 r per day per body dose are not infrequent and exposures of some persons may reach as high as 2 r.[586] The United States Advisory Committee on X-Ray and Radium Protection suggested in 1931 a tolerance dose of 0.3 r per day, and in its revised report in 1936 it recommended 0.1 r per day. This dose was adopted for the operations of the atomic energy plants. Lorenz, on the basis of experimental data, suggested in 1946 that the permissible dose for women who intend to be exposed to radiations for many years should be limited to 0.02 r for eight hours a day. He indicated that the total accumulated exposures should not exceed 100 r to women and 1,000 r to men.[588] Sievert,[589] of the Caroline Institute, Stockholm, proposed in 1947 to fix the safety tolerance dose for general irradiation at 0.01 r per day. The Chalk River Project, in Canada, has recently set its tolerance level at 0.05 r per day.[587] It is obvious from these data that the level of the maximum permissible dose has considerably been reduced during the past decade and that even the presently adopted level may not be entirely safe, to judge from the hematic changes observed among cyclotron and atomic energy workers having an exposure below the adopted level.

Cancer development has only exceptionally occurred after an external irradiation of less than 1,000 r. While the carcinogenic dose usually was delivered in fractionated doses, cancer, as well as leukemia, has followed a single exposure to

† References 572-575.

‡ References 549, 576, and 577.

§ References 578 and 579.

‖ References 584 and 585.

ionizing radiation.[592] Sarcoma of the bone has been observed with as little as 0.5 γ of radium in the entire body.¶ Martland, moreover, stated that if the examination of expired air indicates the presence of 0.1 γ of radium in the body, workers should be removed from the operation. Radon concentrations in air should not exceed 10^{-11} curie per liter at any place at any time. Experimental observations made by several workers (Kaplin, Lorenz) suggest that the tolerance level for very young persons may be even lower.[591] It appears from this evidence, according to Miller,[591] that "there is more than a suspicion that man, especially during early years of his life, can acquire from therapeutic and diagnostic roentgen procedures some of the ingredients of carcinogenesis. It must be kept in mind that neoplasia induced by x-rays would duplicate that occurring spontaneously, and that it would appear at the usual manifestation age of the naturally occurring disease. Therefore direct implication of x-rays in its genesis could only be accomplished by recognizing an over-all increase in the incidence of neoplasia in exposed individuals." Perhaps the British Medical Research Council had similar considerations in mind when it warned against the use of radioactive isotopes in the treatment of children suffering from nonmalignant diseases.[562]

(*b*) Radiation Cancer of the Skin: Reports on the occurrence of occupational and medicinal radiation cancers of the skin have continued to appear. Henry # recorded 31 fatal cases of x-ray cancer of the skin, 17 of them affecting the upper limb, 1 the upper limb and the chest, 1 the chest, 1 the back, and 3 the face. Twenty-five of them were of occupational origin (2 x-ray apparatus makers or testers, 23 roentographers), while the remaining 6 were the result of diagnostic or therapeutic applications of x-rays. In a series of 121 cases of radiation injuries of the skin reported by Teloh and co-workers,[565] there were 34 cases of carcinomas, with 55 individual primary malignant lesions. Additional cases of cutaneous radiation carcinoma involving the fingers and hands of five dentists following occupational exposures to x-rays were published by Mohs.[593] Examples of medicinal cutaneous carcinomas of the skin were placed on record by other investigators (2 cases,[594] 11 cases,[595] 1 case,[596] 20 cases,[597] 1 case,[598] 1 case,[599] 3 cases [560]). Both squamous cell carcinomas and basal cell carcinomas were seen, and not infrequently the lesions were multiple. Both histologic types occasionally occurred in the irradiated skin of the same patient. Several reports mentioned postirradiation cancers of the skin of spindle cell structure, which were interpreted either as spindle cell carcinomas [601] or as fibrosarcomas.*

Henshaw, Snider, and Riley [605] provided experimental evidence on the development of carcinomas and sarcomas of the skin in rats appearing 10 months to 1 year after exposure to P^{32} impregnating a plastic film either in a single massive dose or in repeated small daily doses.

(*c*) Radiation Cancer of the Bones: There have been during the last 12 years many additions to the number of medicinal radiation osteogenic sarcomas either appearing as a consequence of external x-irradiation or resulting from the deposition of bone-seeking radioactive materials. Only one other case of occupational osteogenic sarcoma ending fatally was added to the eight previously observed ones occurring among some 800 luminous-dial painters. In their extensive analysis of

¶ References 589 and 590.

References 411-413.

* References 602-604.

the late effects of internally deposited radioactive materials in man, Aub and co-workers [582] reported three cases of osteogenic sarcoma—two of which developed after the parenteral administration of radium, while a third resulted from the consumption of radioactive water. So far no cases of osteogenic sarcoma among luminous-dial painters have been reported from other countries (Germany,[606] Great Britain [607]). More recent surveys made in American dial-painting establishments showed that there still occurs a disturbingly frequent excessive exposure to radioactive material,† since about 15% of the workers are said to accumulate more than the tolerance dose of radium.

The majority of medicinal osteogenic sarcomas followed upon an external administration of roentgen rays, usually for nonmalignant conditions and involving various bones present in the irradiated field. Cahan and co-workers [610] reported a series of 11 cases of medicinal radiation sarcomas of the bones, all but 1 elicited by the administration of more than 3,000 r and appearing after a latent period of 5 to 22 years (average, 11.2 years). Wolfe and Platt [611] observed two cases of osteogenic sarcoma of the nasal bones following upon an external application of ionizing radiation for epidermal lesions of the skin of the nose. They noted that the entire European and American literature contains about 60 cases of osteogenic sarcoma following upon exposure to roentgen rays or radium therapy. In this total the cases recently recorded by Hatcher,[612] De Young,[613] Tebbet and Vickery,[614] Spitz and Higinbotham,[615] Auerbach and co-workers,[616] and Godwin [594] are not included.

The experimental production of osteogenic sarcomas in mice and rats by the feeding or implantation of naturally or artificially radioactive substances (radium, radon, uranium, Thorotrast, plutonium, Sr^{89}, Ce^{144}, and Pr^{144}) was reported by several investigators.‡

(*d*) Radiation Cancer of the Nasopharynx and Paranasal Sinuses: Recent observations indicate to an increasing degree that the inhalation of radioactive dusts and gases (radon),§ or the direct application of ionizing radiation (x-rays, radioactive substances) to the mucosa of the nasopharyngeal region may elicit carcinomas originating from the irradiated mucosal epithelium. There were among the cancers found in luminous-dial painters three cases of epidermoid carcinomas of the nasal sinuses.[582]

Some concern may be noted as to the possible late appearance of local radiation injury in the nasal, nasopharyngeal, and tonsillar region related to the rather widely used radiation therapy for the control of hyperplastic and chronic inflammatory changes of the tonsils, adenoids, and nasal mucosa, particularly in children with perennial colds and sore throats ‖ or administered to airplane and submarine personnel for alleviating obstructions of the Eustachian tube.¶ This warning is voiced because of the following evidence: Goolden [629] collected from the literature six cases of carcinoma of the deep structures of the neck (laryngopharynx four, larynx one, and thyroid one) following irradiation of the cervical region for benign lesions. To this group he added four cases of his own; Holinger and Rabbett [630] noted three

† References 608 and 609.
‡ References 617-623.
§ References 606 and 624.
‖ References 625 and 626.
¶ References 627 and 628.

similar cases of postirradiation carcinoma of the larynx and pharynx; Ogilvie [631] recorded another one, and van Nieuwenhuyse,[632] three cases.

Apart from the fact that there may exist certain radiation hazards to the physician or medical personnel (finger tips, body) handling the radium applicator during the treatment of nasopharyngeal conditions,[633] the use of doses surpassing 500 r should best be avoided, especially in children, so as to prevent the occurrence of radiation cancers in the exposed tissues some 10 to 30 years later.

(*e*) Radiation Cancer of the Lung: The great rise in the mining, milling, and processing of uranium ores and in the production of radioactive substances has markedly increased the opportunities of inhalation of radioactive gases, vapors, and dusts and of nonradioactive dust contaminated by adsorbed radioactive particles, and thereby has appreciably accentuated the risks of lung cancer from such exposures.# The majority of investigators of the occurrence of lung cancer among the miners in Schneeberg and Joachimsthal favor the view that these neoplasms are the result of an occupational respiratory exposure to radioactive dust and gases.* Since the radioactivity of the Joachimsthal mines is stated to be 30 times the tolerance dose, it is scarcely surprising that in the past more than half of the miners died of lung cancer. Baader [638] recently reported that during the four-year period of 1939 to 1943, 180 cases of cancer of the lung were recognized as of occupational origin in the Joachimsthal area, while there were only a total of 323 miners in 1929. The factually unsupported claim that hereditary factors resulting from inbreeding of the miner population in Joachimsthal played a significant role in determining this excessive liability to lung cancer † is scarcely applicable to the miners of Schneeberg, whose death rate from cancer of the lung has stood at about 75% of all deaths.[643] There is, moreover, no valid evidence indicating that silicosis found in the lungs of some of these miners dying with pulmonary cancer is of etiologic importance.‡

So far, similar observations have not been reported from similar mining and processing establishments in Canada, Congo, and Belgium, which have been in operation for over 20 years. Since the great majority of the mining and milling operations carried on in the Colorado Plateau are not older than 10 years, it is not likely that any appreciable number of lung cancers from these sources may have arisen as yet, although the exposure conditions existing in some of these operations are such that the occurrence of radiation cancers of the lung may be anticipated for the coming years, especially since polonium has been demonstrated in the urine of uranium ore miners of the Colorado Plateau exposed to the inhalation of considerable amounts of radon.[661]

Several attempts have been made during the last decade to produce cancers of the lung in experimental animals by exposing them to the inhalation of radioactive matter. German investigators § reported an abnormally high incidence of pulmonary tumors in mice exposed to the inhalation of radon, while Lisco and Finkel [648] obtained bronchiogenic cancers in the lungs of rats to which Ce^{144} was administered as an aerosol.

References 571, 634, and 635.
* References 636-641.
† References 640-642.
‡ References 641 and 643.
§ References 644-646.

It may be mentioned, moreover, that the occurrence of a bilateral carcinoma of the lung was noted in a woman 18 years after the intravenous injection of Thorotrast.[647] While the lung showing a primary alveolar tumor of mucus-secreting type was nonradioactive, radioactivity and Thorotrast could be demonstrated in the liver and spleen at the time of death. Although there are two additional reports on record in which it is claimed that Thorotrast deposits were the cause of cancers (endothelial cell sarcoma of the liver,[649] spindle cell sarcoma of the kidney [650]), it is more likely that these two tumors formed in proximity to the Thorotrast deposits represent radiation cancers rather than the first-mentioned case of bilateral pulmonary carcinoma, because of the lack of radioactivity of the lung and the rare histologic type of cancer observed. The successful experimental production of sarcomas in rats and mice following the injection of Thorotrast,‖ however, is an important warning against the continued diagnostic use of this chemical in medical practice.¶

There is no valid reason to assume that occupational contact with commercial lead conveys an excessive liability to lung cancer, as proposed by Black.[660]

(*f*) Radiation Leukemia: During the last decade numerous observations have been reported which support the concept that low-level repeated exposures of the whole body or one single massive exposure to ionizing energy may cause or initiate the development of leukemia in man.# Warren,* in 1942, collected a total of 24 cases of alleged radiation leukemia from the world literature and concluded from an evaluation of the evidence that doubt still exists as to the etiologic role of radiation in the development of leukemia in radiologists, but on the basis of experiments on animals, it appeared probable that repeated exposures to small doses of radiation could serve as an exciting or a precipitating cause of leukemia. Despite greatly extended evidence on this subject, Stone,[665] in 1952, still maintained that while leukemia and lymphoid tumors had been produced by ionizing radiation in rats and mice, such an effect was only probable in human beings.

Henshaw and Hawkins [666] showed that the incidence of leukemia in all physicians is almost twice as great as that in the population in general. Dublin and Spiegelman,[667] in a more recent analysis, obtained about the same numerical relation. There were 95 deaths from leukemia and aleukemia among male physicians at ages 25 and over, representing 0.6% of all deaths and giving a death rate per 100,000 of 11.4, in contrast to that of 6.5 among American white males (ratio of leukemia death rates, physicians to white males, 1.75). An even larger series of leukemia cases among physicians evaluated by Peller and Pick [668] gave similar results. Analyzing the deaths among American physicians for the years 1947 to 1951, they found a total of 133 cases of leukemia as a cause of death, i. e., 1.2% of death from all causes and 12.4% of all cancers. Thus, of all fatal cancers among physicians 8.5% were leukemias, as compared with 3.5% in white males. This excessive liability of physicians to leukemia may be due to the fact that not only radiologists, but also other physicians, use x-ray equipment in their practice.

‖ References 651 and 652.
¶ References 653-659.
# References 208-214 and 662.
* References 663 and 664.

The apparent leukemiogenic effect of long-continued total-body irradiation becomes more strikingly apparent in the surveys limited to radiologists and covering periods up to 20 years.† These showed that the incidence of leukemia in radiologists is over nine times as great as in nonradiological physicians. The statistical studies of Peller and Pick [668] revealed, moreover, that dermatologists also have an excessive death rate from leukemia (all physicians, 12.5 per 100,000; radiologists, 69.0; dermatologists, 45.0, and all white males, 9.5). It can be concluded from these data that leukemia may be considered a "calculated risk" of persons using ionizing radiation for medical purposes until improved methods of protection become available.[674]

The existence of causal relationships between such exposures and leukemia received some support from the fact that one of the luminous dial painters died during recent years from leukemia.[582] This observation confirmed a suspicion first voiced by Martland when studying cases of radium and mesothorium poisoning in luminous dial painters. The blood picture in many of his cases was within normal limits, while the bone marrow showed such degrees of hyperplasia and immaturity that "it would seem very likely that a myeloid leukemia could easily develop." It may be mentioned in this connection that more recent hematic studies on luminizers in England revealed, during the early stages of overexposure to radium, absolute and progressive leucopenia, while during later years (1940 to 1947) this condition was conspicuously absent and was replaced by hematic deviations suggesting a hyperstimulative effect.‡ The occurrence of a stimulating effect of chronic exposure to radiation also is apparent from information obtained by Helde [678] on 1,406 persons employed in radiological work, of whom 15 displayed leucocytoses of over 10,000 cells and 46 revealed in their differential count a shift to the left.

A few observations on the occurrence of leukemia in patients subjected to prolonged ionizing radiation provided additional supporting evidence to the thesis advanced.§ It is, however, improbable that such a connection existed between the development of leukemia and leukemoid reactions [681] among 148 cases of polycythemia treated with radiophosphorus by Stroebel and Pease,[680] since such associations are not infrequently observed.‖

The more recent data on the development of radiation injuries among the population of Hiroshima and Nagasaki also indicate that even a single, but massive, exposure to ionizing radiation may favor the development of leukemia. While Brues [685] stated in 1951 that "it may be significant to note that no reports have yet appeared indicating an increased incidence of human leukemia or other malignant disease in the Japanese bombed areas,"¶ it has since become increasingly obvious that a definite increase in the incidence of leukemia among the exposed population groups in Japan has occurred.# The data so far available indicate that there was at least a tenfold increase in the incidence of leukemia among the heavily exposed group in Hiroshima and Nagasaki. It is, at present, uncertain whether the peak of this development has been reached.

† References 669-673.
‡ References 675-677.
§ References 679 and 680.
‖ References 683 and 684.
¶ References 686-688.
# References 689-691.

Additional experimental evidence also has been obtained on mice and rats showing that an adequate exposure to ionizing radiation from various radioactive substances may cause, among other cancerous manifestations, leukemia.* It was shown, moreover, that x-rays exert an additive leukemiogenic effect when administered simultaneously with methylcholanthrene to mice.[696] Because of the widespread contact of the general population with both ionizing radiation and chemical carcinogens, such observations are of special significance in assessing the role and importance of environmental factors in human carcinogenesis.

(g) Radiation Cancer of the Breast and Uterus: The observation of x-radiation has raised the question of the existence of a causal relationship between the two events. Miyamoto [697] reported the occurrence of a carcinoma of the breast in a male roentgenologist whose skin over the affected side of the chest showed evidence of chronic radiodermatitis. A second case of suspected radiation cancer of the breast seven years following a therapeutic administration of x-rays was recorded by Lisa, Pack, and Gioia.[698] There was marked telangiectasia of mammary skin and diffuse firm fibrosis of the affected breast, which contained multicentric mammary cancers.

Claims also have been advanced regarding the causation of endometrial carcinoma by radiation therapy previously administered to the pelvic region for nonmalignant conditions, especially excessive uterine bleeding. Corscaden and Gusberg,[699] in following for 6.7 years 1,100 women who had received a radium therapeutic menopause for benign causes of bleeding, found nine cancers of the uterine corpus and only six of the cervix. Scheffey [700] collected from the literature 71 case reports of uterine cancer developing after irradiation for benign lesions and added 20 of his own. Similarly, Crossen and Crossen [701] reported four cases of endometrial carcinoma in 526 cases of myoma of the uterus previously treated by irradiation. In a series of 805 cases of endometrial carcinoma recorded by Smith and Bowden,[702] there were 39 with previous radiation therapy to the uterus. Of these, however, only eight probably represented postirradiation cancers. There were in a series of 270 cases of cancer of the uterine corpus reviewed by Speert and Peightal [704] at least 21 who had previous irradiation for benign conditions, while of 590 women with ovarian tumors (343 cancers, 247 cystadenomas) 17 had a history of previous pelvic irradiation for benign conditions.[703] The incidence of previous pelvic irradiation among patients with cervical carcinoma, by contrast, was only 0.3%. Speert [704] and Peightal favor the view that irradiation played a significant role in the production of both endometrial and ovarian carcinomas and point out that there is increasing evidence supporting the causation of uterine sarcomas by previous pelvic irradiation, of which they found 32 cases reported in the literature.† In the mouse, ovarian tumorigenesis can easily be obtained by roentgen irradiation of the ovaries.[717] From a critical analysis of the available evidence, the great majority of investigators,[718] however, come to the conclusion that there is no valid proof for the claim that previous exposures of the female sex organs to radiation increase their liability to cancers, with the possible exception of uterine sarcoma.‡

* References 591 and 692-695.

† References 706-716.

‡ References 704 and 705.

Radiation injuries of the fetus resulting from external irradiation of the pregnant uterus have been observed in man and animals.§ The pregnant uterus, therefore, should be shielded so as to protect the embryo and fetus, even in nonpelvic irradiation. For the same reason, radioactive isotopes, such as iodine, should not be administered to pregnant women.‖ Although the occurrence of postirradiation tumors in children of mothers who received pelvic radiation during pregnancy has not been reported, it may be mentioned that Wilson, Brent, and Jordan [723] observed tumors of the brain undergoing spontaneous regression in rat embryos exposed to x-irradiation on the ninth day of gestation.

(*h*) Radiation Cancer of the Thyroid: The extensive use of radioactive iodine in the control of thyroid cancer and hyperthyroidism apparently has as yet not given rise to radiation cancers of this organ or adjacent tissues (trachea, larynx), although such sequelae may be regarded as distinct possibilities. In a study of thyroid cancer of childhood and adolescence, Duffy and Fitzgerald [724] noted that 10 cases gave a history of previous radiation therapy for thymic enlargement. The significance of this observation for the causation of thyroid cancer, however, is uncertain.[725] It has been possible, on the other hand, to produce cancers of the thyroid in rats,¶ as well as cancers of the hypophysis and trachea in mice by the administration of I^{131}.[726] Doniach [735] concluded recently from his experimental production of thyroid tumors with radioactive iodine in rats that the present dose of I^{131} used in the treatment of exophthalmic goiter may eventually prove carcinogenic.

(*i*) Radiation Reactions in Other Organs: In a study of 40 cases receiving radiation therapy of the mouth and oropharynx, Friedman and Hall [729] observed the development of radiation-induced squamous cell metaplasia and hyperplasia of the mucous glands of the cavity, which, when pronounced, might be misinterpreted as a well-differentiated, infiltrating squamous cell carcinoma.

There is no clinical, pathologic, or experimental evidence that therapeutic and diagnostic radiation exposures of the stomach and intestine have resulted in the production of malignant lesions.# Published data also indicate an absence of radiation cancers of the bladder developing on the basis of the rather frequent and severe radiation injuries sustained by this organ, especially in women. That such late sequelae may be possible is suggested by the pronounced cytologic abnormalities of the epithelial lining of the bladder of dogs subjected to x-irradiation.[733] Petrov and Krotkina [734] succeeded in causing cancer of the gall bladder in guinea pigs by implanting glass tubes with radium into the gall bladder lumens.

The sum total of the numerous observations on occupational, medicinal, and environmental radiation cancers cited indicates that civilized and industrialized mankind has entered an artificial carcinogenic environment, in which exposures to ionizing radiations of various types and numerous sources will play an increasingly important role in the production of cancers.

D. Infectious Agents

1. *Schistosomiasis.*—Endemic schistosomiasis produced by Schistosoma haematobium infections, and prevalent among Egyptians, African Negroes, and inhab-

§ References 719 and 720.
‖ References 721 and 722.
¶ References 726 and 727.
# References 730-732.

itants of the countries of the Near East,[747] has causally been related to the high incidence of cancer of the bladder among Egyptians (bilharzial bladder cancer). While this concept has been held also during recent years by the majority of investigators of this subject,* Afifi † objected to its acceptance because of the absence of proper epidemiologic evidence on incidence rate; age, sex, and race distribution, and occupation, and absence of demonstration of a definite carcinogenic agent in the bilharzial ova. The recent data supporting a relationship between vesical schistosomiasis and cancer of the bladder are as follows: Barsoum, according to Afifi,[744] noted that, on the basis of his autopsy findings in Egypt, bladder cancers furnished 22% of all cancers and stood first on the list for cancers by sites. Ward [741] observed, during two years in a Cairo hospital, 130 cases of bladder cancer, the great majority of which were associated with schistosomiasis. Makar [738] found in three Cairo hospitals, among a total of 3,872 cancers affecting the tongue, stomach, colon, rectum, breast, uterus, and bladder, 1,696 vesical neoplasms (43%), as against 3% in an Italian hospital and 11% in a Greek hospital. In addition to this excessive frequency of bladder cancer in association with bilharziasis in Egypt, this parasitic cancer occurs also in younger age groups.‡ The majority of bladder cancers in European and American populations affect persons above the age of 50, while among 360 cases of bladder cancer observed by Makar, only 22% were of this age group. Behairy [736] stated that the usual age of his bladder cancer patients with schistosomiasis was between 20 and 30 years. While the Egyptian observations support the specific infectious origin of bladder cancers in that country, there is a remarkable and disconcerting lack of confirmatory evidence from other regions in which Schistosoma hematobium infections are endemic, and where, in part, the population is racially related to the Egyptians and follows the same religious customs (Iraq).

Higginson [747] mentioned that he found among nine cases of bladder cancer in South African Bantus, two complicated with schistosomiasis. Berman noted § ova of S. haematobium in the bladder of 24 of his cases of primary liver cancer. The claim that cancer of the liver might develop on the basis of a Schistosoma infection (S. haematobium, S. mansoni, or S. japonicum), which is endemic in parts of Africa, South America, and southern Asiatic countries, has lost favor in recent years with most observers. Berman ‖ did not find ova in the liver in any of his cases of hepatic cancer, although bilharzial cirrhosis is frequently encountered on postmortem examination of South African natives. Gelfand [750] found that in Southern Rhodesia cirrhosis and cancer of the liver are as frequent in districts in which bilharziasis is frequent as in those in which it is rare. Hartz,[748] likewise, did not believe that schistosomiasis played a significant role in the production of primary liver cancer among the Chinese in the Netherland West Indies because of the absence of any signs of schistosomiasis in the livers of the five cases he studied. In a series of five cases of cirrhosis of the liver with schistosomiasis studied in New York, and occurring among persons coming from Central America, none had cancer of the liver.[749] Symmers even was reluctant to ascribe the cirrhotic

* References 736-743.
† References 744-746.
‡ References 736 and 738.
§ References 30 and 32.
‖ References 30 and 32.

changes to the presence of ova in the liver, but suggested that these lesions, rather, were attributable to severe dietary deficiencies. Barsoum [754] observed, among 880 autopsies, 160 cases of bilharzial cirrhosis of the liver without a single malignant hepatoma. From their observations among the natives of Katanga and Congo, de Bie and Delville [751] concluded that the presence of bilharzial ova as such in the liver is not the cause of liver cirrhosis, and that there do not exist any causal relations between hepatic schistosomiasis and cancer of the liver, especially since this infection is frequent among the natives, while liver cancer is rather rare among the natives of these regions.

Of equally controversial nature are the claims that rectal schistosomiasis, causing hyperplastic proliferations of the mucosa, represents a significant factor in the development of rectal cancer. Although almost all observations on the established or alleged association between cancer and schistosomiasis originated from foreign, and mainly tropical or subtropical, countries, they have become of a certain importance to the United States because it harbors snails which have been shown to be suitable hosts to the larval form of the worms, thereby providing an essential prerequisite for a potential successful transmission of the infective agent to certain parts of this country. The increasingly frequent residence of members of the American population in countries in which schistosomiasis is endemic, moreover, has resulted in the observation of cases of schistosomiasis (S. haematobium and S. mansoni) in various parts of the United States. Peters, Huntress, and Porter [752] added, in 1945, two cases of this disease to some 35 previously observed in the United States and Canada.[753] In one case, the association of schistosomiasis and cancer of the rectum in a former member of the armed forces who served in the Pacific area during World War II has been the subject of compensation claims against the Federal Government. A more thorough study of such potential interrelations, therefore, has become also for the United States of direct practical importance.

2. *Amebiasis.*—Since opportunities of infection with Endamoeba histolytica were greatly increased for the same reasons, the recent claims or suggestions that intestinal and hepatic amebiasis is causally related to cancers of these organs are of similar scientific and practical significance.¶ Apart from the fact that isolated polypous proliferations of the intestinal mucosa may resemble cancerous growths, there is at present little definite evidence supporting the existence of causal relations between these two conditions.

(*a*) Ulcerative Colitis: This conclusion, however, may not be final—not only because the epidemiologic evidence on this subject is highly defective, but also especially in view of several recent reports connecting chronic ulcerative colitis with an increased liability to carcinomatous developments in the polypoid formations seen often with this condition.# It appears from these reports that the incidence of cancer of the colon and rectum is abnormally high in persons with prolonged chronic ulcerative colitis and that the cancer attack rate rises steeply with the duration of the disease. Many such carcinomas appear to have a multicentric origin, to be of highly malignant character, and to affect persons of younger age than that found in general among patients with colon cancers.*

¶ References 755-758.

References 759-771 and 776.

* References 763-765.

There is, however, no general agreement on the existence of such connections. Some investigators could not discover any evidence of malignant transformations in polypoid growths found with chronic colitis,† while others were unable to demonstrate any excessive incidence of cancer of the lower intestinal tract among persons suffering from chronic ulcerative colitis.‡ Although a final decision on this question must therefore be withheld until more conclusive evidence becomes available, a cocarcinogenic action of the inflammatory and hyperplastic reactions elicited by the factors causing chronic ulcerative colitis in conjunction with specific carcinogenic factors apparently not infrequently present in fecal matter may appear a distinct possibility.

3. *Virus Hepatitis.*—During recent years, evidence has been produced indicating that acute virus hepatitis may be followed by the development of cirrhosis of the liver.§ Since cirrhosis of the liver creates an established increased liability to cancer of this organ, it is not improbable that cirrhosis as a sequela of a virus infection may share such relationships. In fact, two cases of liver carcinoma following upon a viral hepatitis have been placed on record.[780] Mention in this connection may be made of the speculative claim that the hepatic cancers observed among South African Bantus might be caused by a viral agent, because of the demonstration of intracytoplasmic inclusion bodies in the hypophysis.[781]

Additional evidence noting viral infections as precursor conditions of some human cancers was provided by the observation of the development of vulval carcinoma in Bantu women originating in condylomata acuminata (in 4 of 11 cases of vulval carcinoma).[782] Similar findings had previously been reported concerning the appearance of penile carcinoma in condylomata acuminata.[790] The available evidence lends scant support to the hypothesis of a direct viral causation of human cancers.

E. Tobacco Extractives

Friedell and Rosenthal [784] produced additional clinical evidence supporting the concept that chewing tobacco is an etiologic factor in the development of cancer of the mouth.[790] More recently published information indicates that tobacco extractives (alkaloids) [788] may be the causative agent responsible for the excessive incidence of oral cancer among the population of certain parts of India, Indochina, and the Philippine Islands, since the betel quid used in these regions usually contains tobacco as one of its ingredients [787] and because an excessive incidence of oral cancer is absent among those users of betel quids who omit the tobacco (New Guinea, Guam).‖ The present data do not favor the view that the essential carcinogenic factors in the production of the betel cancer might be furnished by the betel leaves containing certain allyl phenols and isomeric propenyl compounds which dimerize under the influence of sunlight and form stilbenols.[789]

F. Dietary Factors

During the past decade, a great deal of new information has been added to the already existing evidence incriminating multiple or single dietary deficiencies and dietary additives or contaminants in the production of human cancer.

† References 772 and 773.
‡ References 774 and 775.
§ References 777-779.
‖ References 785 and 786.

1. *Nasopharynx and Oropharynx.*—Multiple dietary deficiencies are probably responsible for the endemically excessive occurrence of cancer of the oropharynx among the inhabitants of Sweden and Finland residing within the Arctic Circle and living throughout the year on a diet mainly consisting of reindeer meat and salt fish. Only during the short summer months are green vegetables available. As a result of this imbalanced, vitamin-deficient diet, the female members, particularly, develop chronic inflammatory, ulcerative, and hyperplastic reactions of the oropharynx (Plummer-Vinson syndrome). The development of cancers from the oral and nasopharyngeal hyperkeratoses is not infrequently seen during the course of this deficiency state. Ahlbom [791] found this syndrome in 65% of 123 women with cancer of the mouth, pharynx, and esophagus. Martin and Koop [792] likewise maintained that avitaminosis B is the greatest single cause of precancerous oral changes. It is, moreover, likely that the excessive frequency of cancers of the nasopharynx found among Chinese has, as previously mentioned, not a racial but an environmental background.

2. *Liver.*—There exists a good deal of circumstantial and experimental evidence incriminating dietary deficiencies as important causal factors in the production and excessive frequency of primary liver cancer among certain groups of African Negroes, Javanese, and Chinese (Berman ¶). It appears that large parts of the populations of tropical and subtropical Africa and Asia suffer not only from undernutrition but especially also from malnutrition. Severe nutritional disorders often develop in infants after the period of weaning, occasioned by the sudden change to the adult diet, which generally contains little protein and fat and much carbohydrates and roughage. The resulting state of malnutrition, called *kwashiorkor,* causes in the infants retardation of growth, susceptibility to respiratory and other infections, diarrhea, edema, and a unique type of dermatosis known as "crazy-pavement dermatosis," or "infantile pellagra."# Pathologic lesions include the disappearance of zymogen granules from the pancreatic acini and the accumulation of fat in the periphery of the hepatic lobules. The survivors of these serious dietary disturbances also during adult life usually consume a diet deficient in protein, specific amino acids, and vitamins, particularly vitamin A and the vitamin B complex. The diet of the South African Bantus, among whom liver cancer is frequent, mainly consists of a thick cornmeal mush (mealie meal) plus small amounts of fermented milk. Meat is eaten rarely.[795] Cirrhosis of the liver among these Negroes is frequent (found in four out of five autopsies performed on male Bantus at the Johannesburg General Hospital).* It stands to reason that the cirrhosis of the liver seen in chronic drunkards also is the result of nutritional disturbances and not of alcohol toxicity, because drunkards often substitute alcohol for an adequate meal and suffer from dietary protein and vitamin deficiencies.†

Adequate experimental evidence, moreover, attests the fact that cirrhosis, as well as cancer of the liver, can be produced in rats placed on a nutritionally deficient diet. Its relation to cirrhosis was demonstrated by South African investigators,‡

¶ References 30 and 32.
# References 793 and 794.
* References 795-797.
† References 798, 799, and 804.
‡ References 795, 800, and 801.

who fed maize meal porridge (mealie pap) and sour milk to rats. In addition to various disturbances of growth and of the skin and teeth, the rats developed fatty degeneration and, finally, cirrhosis of the liver. Similar hepatic lesions were observed by Webster [802] and György and Goldblatt [803] in rats placed on a deficient diet (protein, fat). Experiments of several investigators, moreover, have shown that such cirrhotic changes can be elicited in the liver of rats and mice when they are placed on a choline-deficient diet § or when the intestinal utilization of dietary choline is interfered with by the simultaneous oral administration of bentonite.[808]

The etiologic relationship of this dietary cirrhosis of the liver to hepatic neoplasia was established by the demonstration of hepatomas in rats and mice thus treated.‖ The hepatomagenic effects upon the livers of rats, associated with diets deficient in a related nutrient, methionine, of feeding a biologic antagonist, ethionine (alpha-amino-gamma-ethylthiobutyric acid) [811] further strengthens the evidence that dietary hepatotoxic deficiencies, like other chemotoxic, radiotoxic, and parasitotoxic agents, may elicit not only cirrhosis but also carcinoma of the liver.¶ The existence of such causal relations for the primary cancer occurring among African and Asiatic population groups is suggested by the fact that cancers of the liver are unusually frequent among relatively young persons, i. e., among persons who have reached the end of the usual latent period of environmental cancers (10 to 30 years), which in this particular case started with infancy or childhood. These cancers behave, in this respect, like the scrotal cancers among English climbing boys of the last century or the penile cancers among noncircumcised populations with defective personal hygiene.

Recent epidemiologic observations, moreover, indicate that the development of cancers of several other organs may causally be related or aided by prolonged nutritional disturbances. Thus, Marsden [815] reported the unusually high frequency among the Malayans and Chinese in Malaya of salivary gland tumors, involving mainly the submaxillary gland, and not, as in Europe and America, mainly the parotid gland, although these tumors were uncommon among Europeans and Eurasians residing in Malaya. Salivary neoplasms constituted 4.5% of all neoplasms seen in the Pathological Institute of Malaya. Malignant malnutrition is prevalent among the infants and children of the poorer classes in Malaya, and this state is not infrequently associated with a swelling of the salivary glands (Malayan school children 20%, Chinese 10%, Indian 5%). Similar observations were made in 1947 in South Africa by Gillman, Gilbert, and Gillman,[816] who reported that such enlargements are caused by cellular glandular hypertrophies, fat deposition, and fibrosis, and resemble similar changes seen in the liver and pancreas with starvation.

3. *Breast.*—During recent years data have accumulated which indicate that certain metabolic and excretory endocrine disturbances associated with malnutrition and undernutrition, apparently related to starvation, produced functional impairments and cirrhotic changes of the liver and may be involved in the development of epithelial hyperplasias and cancers of the breast and uterus [817] and in the inhibition of hypertrophy of the prostate.# There exists, apparently, a general

§ References 805 and 806.

‖ References 805, 809, and 810.

¶ References 812-814.

References 818 and 819.

statistical association between the incidence of cirrhosis of the liver and cancer of the male breast in populations (Greece, Africa) suffering from chronic states of semistarvation.[827] Since cancer of the male breast has developed sometimes on the basis of a gynecomastia,* it is of interest to note also that gynecomastia in males is frequently met in malnourished population groups, such as the Greeks during World War II † and African Negroes,‡ and was observed during World War II among American and English prisoners kept on a highly deficient diet [828] in Japanese prison camps.§

These hyperplastic reactions of the breast were in general attributed to disturbances of the liver metabolism of steroids, especially estrogens, and were accompanied or preceded in these and other cases of poststarvation or cirrhotic gynecomastia by other symptoms of hormonal imbalance, loss of libido, sexual impotence, female type of distribution of pubic hair, and testicular insufficiency.‖ There exist a number of other observations attesting the relation of gynecomastia in males to cirrhosis of the liver ¶ or primary cancer of the liver.[836] It is still controversial whether these mammary reactions are related to a defective destruction or inactivation of estrogens by a functionally insufficient liver, resulting in an accumulation of estrogens in the organism,[838] such as is seen with acute infectious hepatitis,[840] or to interfered excretion of estrogens by the cirrhotic organ,[839] or to an estrogen-androgen imbalance, indicated by the frequently coexisting testicular atrophy.#

Mention may be made in this connection of observations on the apparently negative relation of liver function to cancer of the female breast. Tagnon and Trunnell [842] and Evans and co-workers [844] were unable to demonstrate significant deviations in liver function in women with operable cancer of the breast from that seen in normal women, while Hall and Sun [843] noted from necropsy studies that it appears unlikely that portal cirrhosis influences the development of carcinoma of the breast and uterus.

4. *Uterus.*—It is likewise uncertain whether there is a causal connection between cirrhosis of the liver associated with hyperestrinism and endometrial carcinoma. While Speert [845] felt that his observations demonstrated such interrelations, Brewer and Foley [846] found that there was no increased frequency of endometrial carcinoma in patients with hepatic cirrhosis.

There exists, at present, no sound evidence by which the contradictory observations and interpretations can wholly be resolved. It is, however, possible that the male organism does not handle estrogens and react upon estrogenic disturbances in the same way as the female one, that associated nutritional deficiencies, with their organic functional and anatomic sequelae, provide additional variations in reactivity to hormonal cellular growth stimuli, and that our criteria of and methods for demonstrating hormonal deviations in their relation to malignant growths are inadequate.

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* References 817, 821, and 822.
† References 820 and 827.
‡ References 13, 825, and 826.
§ References 823 and 824.
‖ References 829 and 837.
¶ References 830-835.
# References 831 and 841.

G. Dietary, Occupational, Cosmetic, and Medicinal Estrogens

The growing medicinal use of various natural and synthetic estrogen preparations, their inclusion in cosmetics, and their commercial employment in the fattening of fowl may aid in the elucidation of the role which endogenous or exogenous hyperestrinism may play in the initiation and development of cancers of various organs. In addition to these sometimes not inconsiderable and prolonged exposures of human beings to exogenous estrogens, there exists the possibility of their receiving significant amounts of estrogens in the diet through the cumulative effect of small quantities of estrogens normally contained in various foodstuffs, such as milk, eggs, lobsters, liver, potatoes, yeast, honey, sage, some types of clover, and grasses.*

Additional sources of dietary exposure to estrogens by the general population may be found in the implantation of estrogen pellets into the subcutaneous tissue of the neck of fowl for fattening and tenderizing purposes. An individual dose of 15 mg. of diethylstilbestrol to be implanted once or twice beneath the head is recommended for this type of chemical caponization. Practical experience has shown that the farmers and poultry men do not always follow such instructions, and implant several pellets at a time or insert them at other sites than the neck, such as the breast, i. e., parts which are regularly consumed. Since biologic activity remains in these pellets for a period of up to six weeks, it is advised not to market chemically caponized fowl before the elapse of this time.[846,847] That such practices may lead to an ingestion by unsuspecting members of the general population of not inconsiderable amounts of estrogenic material is obvious.

Additional uncontrolled or ill-controlled environmental exposures to estrogens are related to the use of estrogen-containing cosmetic and dermatologic preparations, such as creams, ointments, and lotions, for topical application to the skin or the breast,† which has resulted when prolonged in some cases in swelling of the breast and endometrial glandular hyperplasia. Similar observations as to gynecomastia have been reported in male estrogen manufacturers, who may absorb these chemicals by skin or respiratory contact.‡ Such findings have been made among employees of English, as well as American, pharmaceutical companies. In addition to glandular hyperplasia of the breast tissue, there has been, in at least one of the American cases, the development of bilateral fibroadenomas, necessitating the amputation of both breasts.

Upon such uncontrolled exposures from multiple environmental sources, a medicinal administration of estrogens for the treatment of menopausal symptoms, endometriosis, and hypogenitalism may be superimposed at times. It is known that an excessive or prolonged administration of estrogens to postmenopausal women may produce uterine bleeding and endometrial hyperplasia.§ Although it is known that there occurs a gradual transition from such hyperplastic changes into definitely malignant ones [863] in the development of endometrial carcinoma, the number of such cancers allegedly attributable to an excessive estrogen therapy so far published is stated to number about nine.‖ The cancers usually appeared 5 to 17 years

* References 840 and 847.
† References 848-853.
‡ References 854-858.
§ References 859-862.
‖ References 864-867, 873, and 876.

after the start of therapy. This appears to be an adequate latent period, considering that the duration of endometrial carcinoma in situ has been estimated to be six to seven years.[875] In commenting on such claims, many gynecologists seem to feel that, in view of the widespread use of estrogens for many years in medical practice, the apparent absence of endometrial carcinomatous sequelae in large series of women thus treated, and the use of doses in women much lower than those which have produced cancers of various organs in experimental animals, there is little indication that under ordinary therapeutic conditions estrogen therapy carries a distinct cancer hazard for the average patient.¶ However, a note of warning is voiced by others, since there is, in their opinion, the possibility that such untoward reactions might ensue in some genetically predisposed persons or in persons previously exposed to appreciable amounts of exogenous or endogenous estrogens.#

The probable existence of causal interrelations between hyperestrinism and endometrial carcinoma is strongly suggested by the fact that estrogen-producing ovarian tumors are as a rule associated with endometrial hyperplasia, endometrial polyps, or carcinoma (carcinoma of uterine corpus in 18 to 20% of granulosa cell tumors).* In contrast, endometrial cancer is practically nonexistent in surgical castrates, but not in radiation castrates.† It is apparent from these observations that hyperestrinism, irrespective of its endogenous or exogenous origin, when prolonged and highly excessive, may either predispose to or cause endometrial cancer.

In analogy to the repeatedly and definitely demonstrated carcinogenic effect exerted by estrogens on the development of mammary cancer in certain selectively inbred strains of mice, claims have been advanced as to a similar action of estrogens in the causation of breast cancer in women.‡ However, the evidence relating to such allegations is controversial. Only a few cases of breast cancer in women have been reported in which, presumably, the tumors developed after prolonged medication with these chemicals.§ In view of the relatively high "normal" incidence of breast cancer in women, such observations, moreover, are bound to have only restricted value in demonstrating such relations because of the unavoidable factor of coincidence.

Of distinctly greater significance, on the other hand, is the growing number of unilateral and, often, bilateral carcinomas of the breast which developed in males given large amounts of estrogens for the control of cancer of the prostate. There is at present on record a total of 17 such cases of breast carcinoma in males, variously diagnosed as primary breast tumors or as metastatic growths of the prostate cancer.‖ Doubtless, additional cases of breast cancer of this origin have remained unreported. While an estrogenic causation of such mammary cancers was accepted by Bauer,[914] and considered as highly suggestive by Hertz,[915] as possible under special conditions by Butenandt,[916] others believe that the tumors are secondary deposits of the prostatic cancers which localized in an estrogenically hyperplastic

¶ References 860, 868, 877, and 878.

References 861, 863, 869-874, 876, and 877.

* References 840, 873, and 879-891.

† References 861 and 889.

‡ References 840 and 892.

§ References 893-895.

‖ References 896-912. Van Winkle, W., Jr.: Personal communication to McClure and Higgins.[904]

breast.¶ The occasional occurrence of simultaneously developing primary cancers in the breast and prostate [915] or the rare formation of a mammary metastasis of a prostate cancer [917] which did not receive estrogen medication, justifies some degree of reservation in concluding that estrogens have been shown to induce mammary carcinoma in the human male.[915] Twombly,[905] moreover, showed that the carcinomatous tissue of the breast in one of these cases showed histochemically a high acid phosphatase content, similar to that of the prostatic cancer. Zondek [918] advanced the fallacious argument that a carcinogenic action of estrogens is precluded by the fact that these chemicals have been used for the treatment of cancers of the breast and prostate. He completely disregards the well-established fact that a considerable number of our presently or formerly used anticarcinogenic agents (ionizing radiation, arsenicals, benzene, some of the mustards) display considerable carcinogenic properties.

In favor of an estrogenic origin of these breast cancers in males, it may be argued that their occurrence was noted only after the introduction of the estrogen therapy of prostate cancers, that about 25% of them show a bilateral character, that gynecomastia usually preceded their development, and that mammary metastases of prostate cancers are apparently very rare, since this site is not even mentioned in the comprehensive monograph on metastases by Walther.[920]

Considering the fact that even in experimental animals (mice, rats) breast cancers can be elicited by prolonged estrogen treatment only in especially susceptible strains,# it may be possible that such reactions in the human also occur only in constitutionally predisposed persons. Because of our inability to predetermine such persons and to ascertain their proportion in the general population, as well as in the patient population, it seems to be highly desirable to restrict prolonged and severe environmental, occupational, and medicinal exposures to estrogens to conditions in which such exposures are justified and which permit adequate control of the exposed persons.

These considerations are appropriate also because it is uncertain whether or not estrogens may elicit in man cancers at other sites (bladder, kidney, leukemia) similar to those caused by these chemicals in mice and rats.*

¶ References 899, 902, 904, and 911.

References 921-923.

* References 840 and 924-927.

(To be Concluded)

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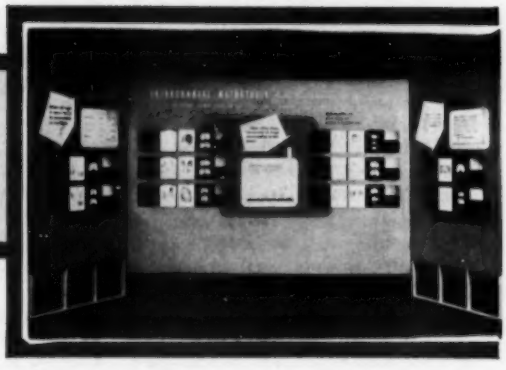
Scientific Exhibits



INTRACRANIAL METASTASIS FROM CARCINOMA OF THE LUNG

BELA HALPERT, M.D.
WILLIAM S. FIELDS, M.D.
AND
MICHAEL E. DE BAKEY, M.D.
HOUSTON, TEXAS

ALTHOUGH it is generally conceded that metastasis to the brain from carcinoma of the lung is of frequent occurrence, information has not been available as to how often it occurs in a substantial number of consecutive cases. During a period of three and one-half years exactly 100 necropsies were performed on patients with carcinoma of the lung, and in 92 of these the brain was included in the examination. We undertook an analysis of these cases to provide answers to the following specific questions: What cell type of carcinoma is most likely to metastasize to the brain? How often does carcinoma of the lung metastasize to the brain? Why should there be a thorough neurological examination of patients with carcinoma of the lung?

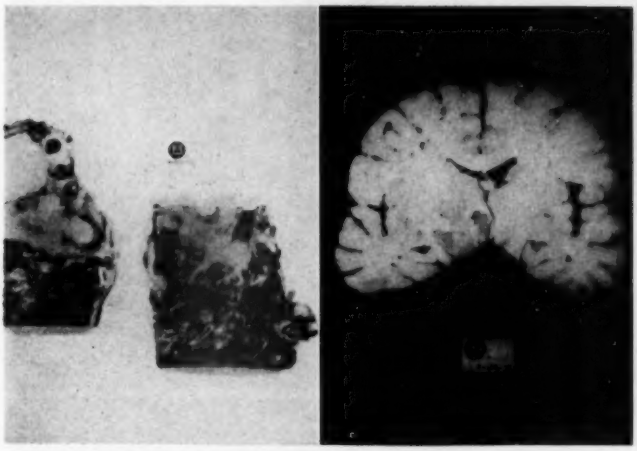
All the patients were men; 85 were white, and 15 were Negro. They ranged in age from 23 to 76 years, and 76 were in the sixth decade of life or older. Intracranial metastasis had occurred in 30 of the 92 patients whose brains had been examined at necropsy; of these, 2 were in the third decade, 3 in the fourth, 4 in the fifth, 14 in the sixth, and 7 in the seventh.

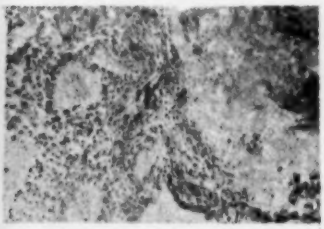
From the departments of Pathology, Psychiatry and Neurology, and Surgery, Baylor Univerity College of Medicine, and the Veterans Administration Hospital.

Shown as a scientific exhibit of the Section on Pathology and Physiology at the 103rd Annual Meeting of the American Medical Association, San Francisco, June 21-25, 1954.

What cell type is more likely to metastasize to the brain

Squamous cell	19	55	34.5%
Reserve cell	8	23	34.7%
Columnar cell	2	6	33.3%
Mixed cell	1	8	12.5%
TOTAL	30	92	





Carcinoma, squamous cell, of lung, metastatic in brain; ×60

F. P., 50, admitted 4/11/52, died 5/1/52
Symptoms: pulmonary, 9 months; C. N. S., 6 weeks
Operation: biopsy, bronchoscopic, 4/15/52
Clinical Impression: carcinoma of lung with cerebral metastasis

The left lung weighed 1,160 gm. It contained a neoplastic mass 8 cm. in diameter in the upper lobe emanating from the bronchial branch.

The brain weighed 1,615 gm. On frontal cut surface 2 cm. posterior to the optic chiasm there was a neoplastic area 4 × 3 cm. in the left thalamus that pushed the third ventricle to the right.

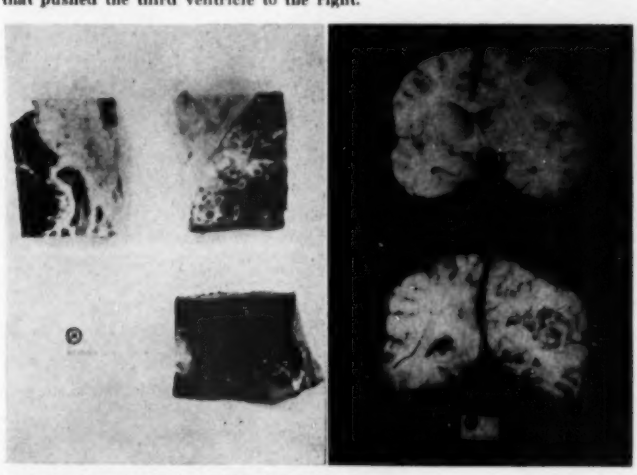
ANATOMIC DIAGNOSIS:

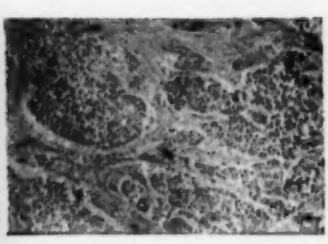
Carcinoma, squamous cell, of lung, left, upper lobe, with extension to pleura, pericardium and heart, and metastasis to hilar lymph nodes and cerebral hemisphere, left

Bronchitis and bronchiolitis, chronic and acute

Nephrosclerosis, arterial

Cardiac hypertrophy





Carcinoma, squamous cell, of lung, metastatic in brain; × 60

C. F. P., 28, admitted 1/2/52, died 8/14/52
Symptoms: pulmonary, 12 months; C. N. S., 3 weeks
Operation: biopsy, supraclavicular lymph node, 1/5/52
Clinical Impression: carcinoma of lung with cerebral metastasis

The right lung weighed 900 gm. In the upper lobe near the hilus there was a nodular neoplastic mass 6 cm. in diameter.

The brain weighed 1,625 gm. In the region of the infundibulum there was a neoplastic mass 1.5 × 1.2 cm. compressing and distorting the optic chiasm. On frontal cut surfaces 6 cm. posteriorly there was a globular neoplastic mass 3 cm. in diameter in the right hemisphere.

ANATOMIC DIAGNOSIS:

Scar of operation: biopsy of supraclavicular lymph node, right (S-21-52)

Carcinoma, squamous cell, of lung, right, with metastasis to regional lymph nodes, mediastinum, suprarenal gland, and cerebral hemispheres

Fibrous pleural and pericardial adhesions

Pneumonia, focal, bilateral

Ancient scar of operation: appendectomy

H. R. L., 37, admitted 12/3/49, died 9/25/50

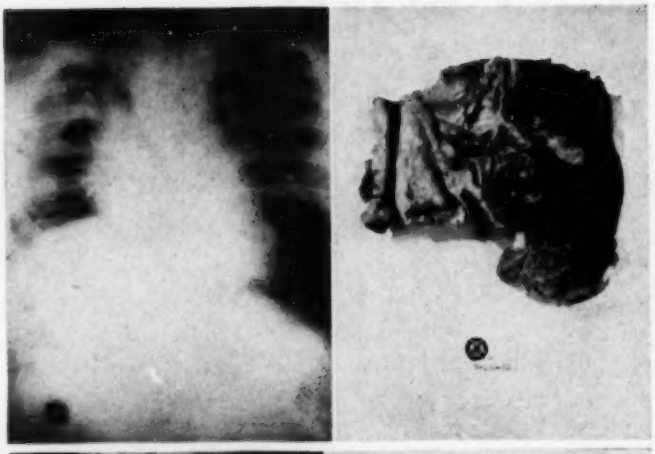
Symptoms: pulmonary, 13 months; C. N. S., none

Operation: biopsy, supraclavicular lymph node, 2/3/50

Clinical Impression: carcinoma of lung, right, with generalized metastasis

The right lung weighed 480 gm. Emanating from the upper bronchial branch there was a neoplastic mass 3 cm. in diameter.

The brain weighed 1,480 gm. On frontal cut surfaces of the cerebral hemispheres at the level of the optic chiasm there was a neoplastic mass 2 × 1.5 cm. in the left cerebral hemisphere in the region of the globus pallidus. On the cut surfaces 2 cm. posteriorly there was another nodule, 1 × 0.5 cm. in the corpus callosum on the right.



L. M. McC., 39, admitted 6/30/50, died 12/5/50

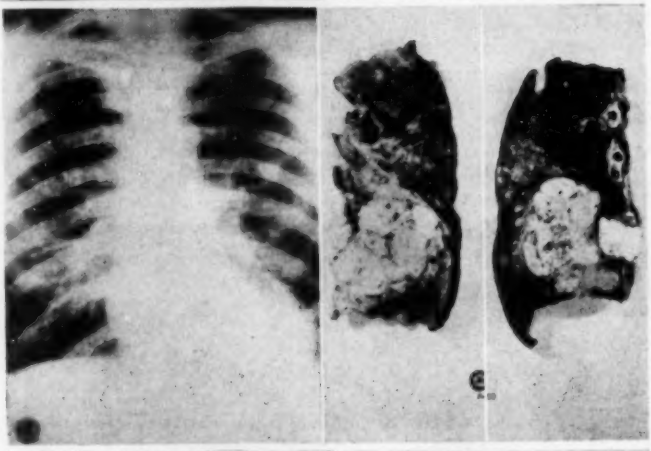
Symptoms: pulmonary, 7 years; C. N. S., 5 weeks

Operation: craniotomy, left frontoparietal, 9/25/50

Clinical Impression: carcinoma of lung with cerebral metastases

The left lung weighed 650 gm. In the lower lobe there was a neoplastic mass 7 cm. in diameter emanating from the lower bronchial branch.

The brain weighed 1,650 gm. On frontal cut surfaces of the cerebral hemispheres posterior to the optic chiasm there was a neoplastic mass 3.5 × 3.5 cm. in the right temporal lobe. There were also neoplastic nodules in both occipital lobes.



C. B. G., 56, admitted 11/24/51, died 12/26/51

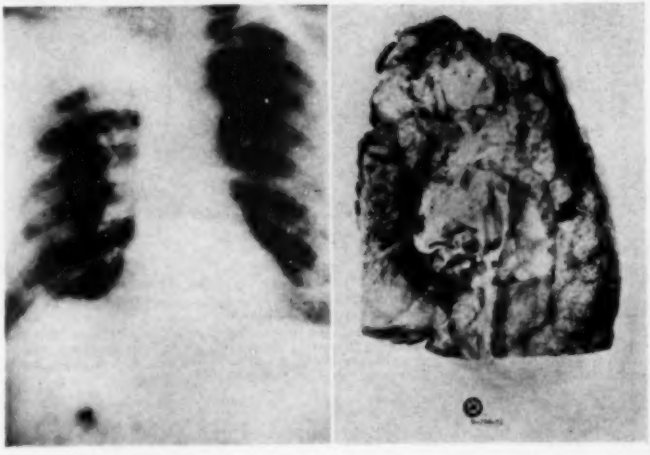
Symptoms: pulmonary, none; C. N. S., 10 weeks

Operation: none

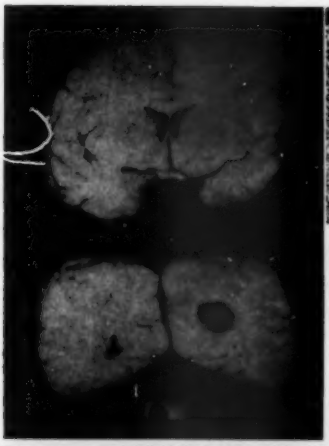
Clinical Impression: carcinoma of lung with metastasis to cerebral hemisphere, right

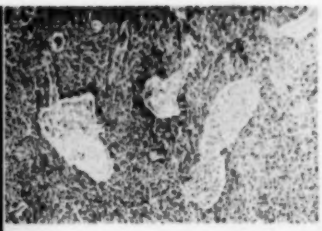
The right lung weighed 1,340 gm. Emanating from the upper bronchial branch there was a neoplastic mass 5.5 cm. in diameter. This was contiguous with a cavity 7 cm. in diameter, containing purulent material.

The brain weighed 1,725 gm. On frontal cut surfaces just posterior to the optic chiasm there was a neoplastic mass 3 × 2.8 cm. in the right frontal lobe. There was a neoplastic nodule 2 × 4 cm. in the left parieto-occipital region.



How often does carcinoma of lung metastasize to the brain





Carcinoma, squamous cell, of lung, metastatic in brain; × 60. The appearance and pattern closely resemble reserve cells.

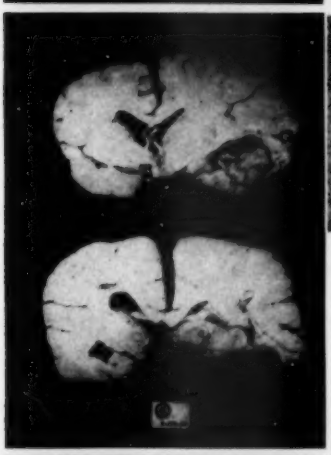
ANATOMIC DIAGNOSIS:

Scar of operation: biopsy of supraclavicular lymph node, right (S-154-50)

Carcinoma, squamous cell, of lung, right, with metastasis to regional and retroperitoneal lymph nodes, lung, left, liver, pancreas, suprarenal glands, kidneys, and cerebral and cerebellar hemispheres

Ascites and hydrothorax, bilateral

Dermatitis of chest wall (irradiation)



Carcinoma, squamous cell, of lung, metastatic in brain; × 60.

ANATOMIC DIAGNOSIS:

Scar of operations: craniotomies for carcinoma, metastatic, parietal region, left (S-1036-50); decompression (S-1449-50); bronchial biopsy (S-1010-50)

Carcinoma, squamous cell, of lung, left, lower lobe, with metastasis to regional lymph nodes and cerebral hemispheres

Atrophy of extremities, right

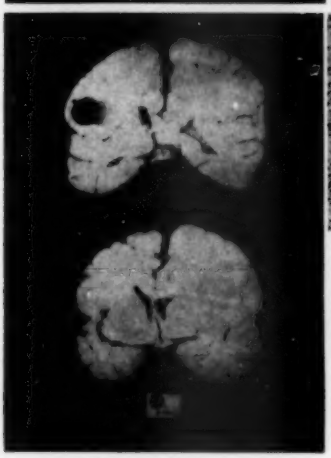
Bronchitis, chronic and acute, with bronchiectasis, lower lobe, left

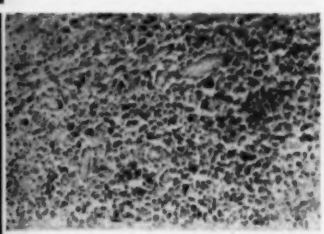
Emphysema, bilateral. Emaciation

Ancient scar of operation: appendectomy

Azygos lobe of lung, right

Accessory spleen





Carcinoma, squamous cell, of lung, metastatic in brain; × 60

ANATOMIC DIAGNOSIS:

Carcinoma, squamous cell, of lung, right, with metastasis to cerebral hemispheres

Fibrous pleural adhesions, bilateral

Chronic passive congestion of viscera with pneumonia, focal, organizing, right

Emaciation

Cholecystitis, chronic, with cholesterolosis and petrified papillomata of gall-bladder mucosa

S. M. C., 62, admitted 10/24/50, died 7/13/51

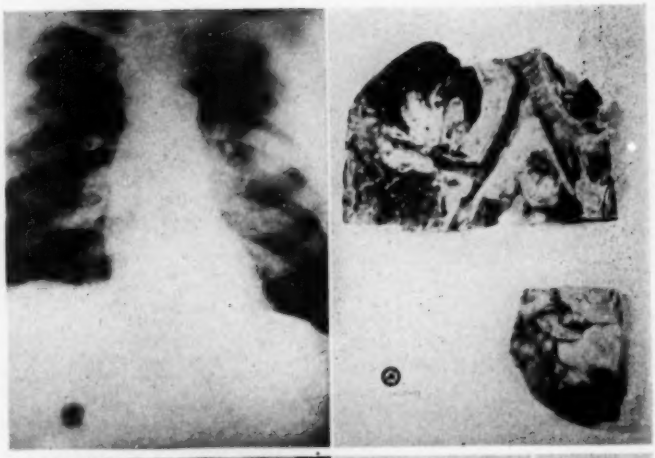
Symptoms: pulmonary, none; C. N. S., 18 months

Operation: none

Clinical Impression: Marie's cerebellar ataxia — parenchymatous cerebellar degeneration; carcinoma of lung with visceral metastasis

The lungs together weighed 2,100 gm. Emanating from left upper bronchial branch there was a neoplastic nodule 2.5 × 1.5 cm. The neoplastic infiltration extended along the left bronchus to the right bronchus.

The brain weighed 1,320 gm. On frontal cut surfaces of the cerebral hemispheres 4 cm. posterior to the frontal poles there were two neoplastic nodules, 2 and 1 cm. in diameter, in the left frontal lobe. There was a similar nodule in the right temporal lobe. A neoplastic mass 1.7 × 1.2 cm. was in the anterior portion of the vermis cerebelli.



J. F. D., 61, admitted 6/8/51, died 12/4/51

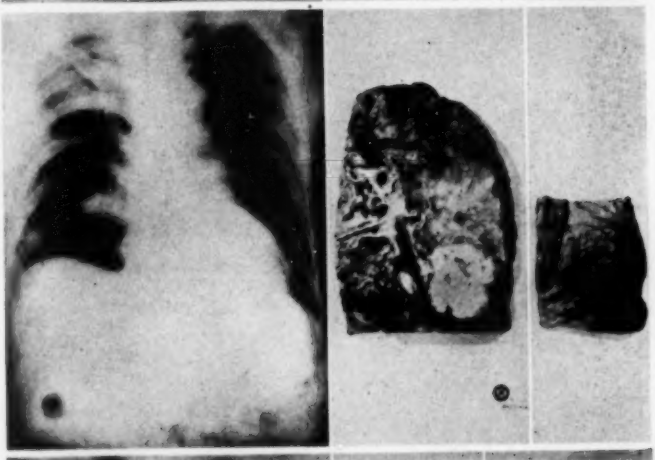
Symptoms: pulmonary, none; C. N. S., 6 months

Operation: craniotomy, right parietal, 6/20/51

Clinical Impression: intracranial neoplasm, metastatic, probably from lung

The right lung weighed 860 gm. Emanating from the lower bronchial branch there was a neoplastic mass 5 cm. in diameter.

The brain weighed 1,480 gm. On frontal cut surfaces just anterior to the splenium of the corpus callosum there was a neoplastic mass 5 × 5 cm. in the right parietal area. A nodule 1.5 cm. in diameter was located in the left occipital lobe.



A. P. McB., 65, admitted 11/26/51, died 12/25/51

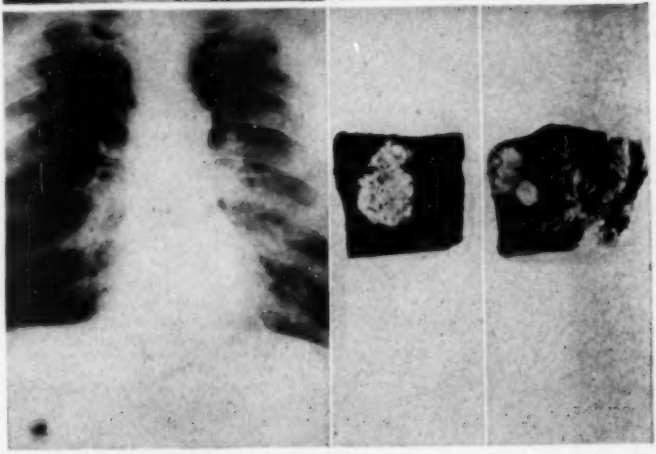
Symptoms: pulmonary, none; C. N. S., 7 weeks

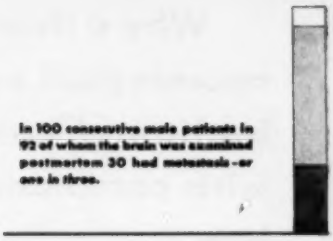
Operation: none

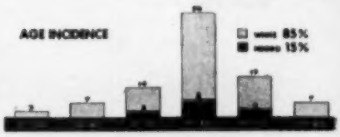
Clinical Impression: carcinoma of lung, left, with metastases to cerebellar and cerebral hemispheres

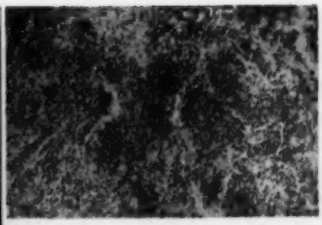
The left lung weighed 650 gm. In the upper lobe there was a neoplastic mass 5 × 5 cm.

The brain weighed 1,480 gm. There was a neoplastic mass 5 × 3.5 cm. in the left cerebellar hemisphere. There was no neoplastic involvement elsewhere. Marked internal hydrocephalus was noted.









Carcinoma, reserve cell, of lung, metastatic in brain; × 60

ANATOMIC DIAGNOSIS:

Scar of operation: biopsy of lymph node, cervical, left (S-791-51)

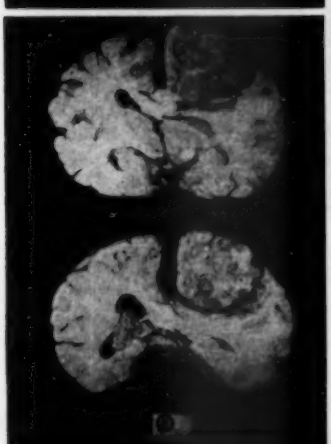
Carcinoma, reserve cell, of lung, left, upper lobe, with extension to lung, right, and pleurae

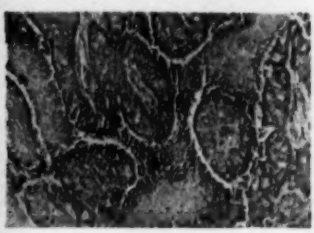
Metastasis to lymph nodes, skin, heart, spleen, liver, pancreas, suprarenal glands, kidneys, prostate, thyroid gland, hypophysis, dura, cerebral hemispheres and vermis cerebelli, ileum, colon, and peritoneum, with compression of ureter, right, and hydronephrosis

Bronchitis and bronchiolitis with pneumonia, focal, bilateral

Emaciation with fatty change of liver

Scar of operation: subtotal resection of stomach for ulcer (S-1670-50) and gastrojejunostomy





Carcinoma, columnar cell and squamous cell, of lung, metastatic in brain; × 60

ANATOMIC DIAGNOSIS:

Scar of operation: craniotomy, right, for carcinoma, metasatic to brain (S-1012-51)

Carcinoma, columnar cell and squamous cell, of lung, right, with metastasis to regional lymph nodes and cerebral hemispheres

Chronic passive congestion of viscera

Pneumonia, focal, bilateral, with abscesses

Fibrous pleural adhesions, bilateral

Carcinoma, squamous cell, of lung, metastatic in brain; × 60

ANATOMIC DIAGNOSIS:

Carcinoma, squamous cell, of lung, left, upper lobe, with metastasis to cerebellum, left

Pneumonia, focal, with abscesses of lung, right

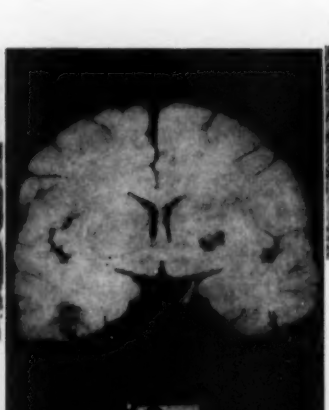
Fatty change of liver, slight

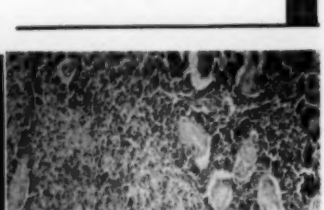
Hyperplasia of prostate

Ancient scar of operation: gastrojejunostomy

Why a thorough neurological examination of patients with carcinoma of lung . . .

Particularly essential when surgical intervention with curative intent is contemplated. Neurological symptoms may precede pulmonary symptoms.
(10% of this series)





Carcinoma, reserve cell, of lung, metastatic in brain; × 60

ANATOMIC DIAGNOSIS:

Carcinoma, reserve cell, of lung, right, with metastasis to regional lymph nodes, pancreas, suprarenal glands, cerebral hemispheres, and medulla oblongata

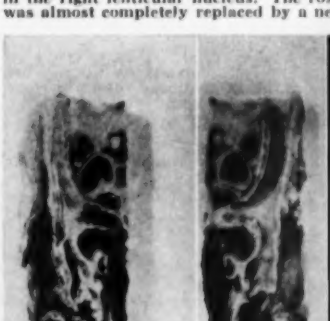
Chronic pulmonary tuberculosis, right, lower lobe

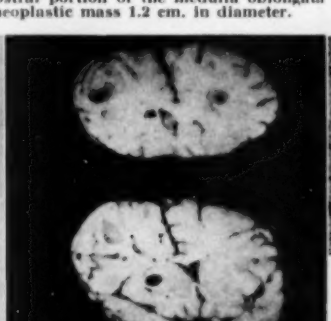
Bronchiectasis, bilateral

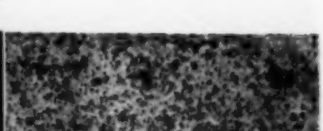
P. H., 44, admitted 10/17/51, died 1/10/52
Symptoms: pulmonary, 12 months; C. N. S., 1 month
Operation: biopsy, bronchoscopic, 11/2/51
Clinical impression: carcinoma of lung; pulmonary tuberculosis

The right lung weighed 900 gm. Emanating from the right main bronchus there was a neoplastic mass 7.5 × 5.5 cm.

The brain weighed 1,135 gm. On frontal cut surfaces of the cerebral hemispheres just posterior to the optic chiasm there was a neoplastic mass 2 × 1.5 cm. in the left temporal lobe. A similar nodule, 1 × 1 cm., was in the right lenticular nucleus. The rostral portion of the medulla oblongata was almost completely replaced by a neoplastic mass 1.2 cm. in diameter.







Carcinoma, squamous cell, of lung, metastatic in brain; × 60

ANATOMIC DIAGNOSIS:

Scar of operation: segmental lobectomy, left upper lobe, for carcinoma of lung (Scott & White Clinic, Temple, Texas, 7-4-50)

Recurrent carcinoma, squamous cell, of lung, left, with hemothorax and metastasis to lung, right, pleura, diaphragm, liver, suprarenal glands, cerebral hemispheres and vermis cerebelli

Emphysema of lung, right

Chronic passive congestion of viscera with hydrothorax, right

Pneumonia, focal, right

Hemorrhage into gastrointestinal tract

Cortical adenoma of suprarenal gland

Prostatitis, chronic

C. W. D., 54, admitted 10/18/50, died 12/11/50
Symptoms: pulmonary, 11 months; C. N. S., none
Operation: lobectomy, left upper lobe, 7/4/50
Clinical impression: carcinoma of lung, left, with metastases

The left lung weighed 600 gm. Emanating from the upper bronchial branch there were several neoplastic nodules up to 2 cm. in diameter

The brain weighed 1,830 gm. On frontal cut surfaces just anterior to the optic chiasm there was a neoplastic mass 3.5 × 2.5 cm. in the right frontal lobe. A similar mass 1.5 × 1.5 cm. was in the right caudate nucleus. On the cut surfaces 4 cm. posteriorly there was a neoplastic mass 2 × 1.5 cm. in the left thalamus. There was a nodule 2 × 1 cm. in the inferior portion of the cerebelli.

News and Comment

Death Notice.—Miss Maud Slye died in Chicago on Sept. 17, 1954, at the age of 75. She had retired from the University of Chicago in 1945, after forty years of investigations, particularly in collaboration with Dr. H. Gideon Wells and Miss Harriet F. Holmes, on the genetics and pathology of cancer in mice. These investigations had a marked influence on the development of cancer research. Many thousands of tumors were produced and classified microscopically. She received the Gold Medal of the American Medical Association in 1914, the Ricketts Prize in 1915, and the Gold Medal of the Radiological Society of North America in 1922.

Death.—Dr. Stuart Graves, Dean Emeritus and Professor Emeritus of Pathology at the Medical College of Alabama, Birmingham, died on July 14, 1954, at the age of 75. Dr. Graves was Professor of Pathology at Alabama from 1928 to 1947. Previously he had been Professor of Pathology and Bacteriology at the University of Louisville School of Medicine, as well as dean. He was a fellow of the American College of Physicians and had been Acting Assistant Surgeon for the United States Public Health Service. He was given the LL.D. degree from the University of Alabama in 1937.

Death Notice.—Dr. Donald Charles Beaver, Clinical Professor of Pathology at Wayne University College of Medicine, Detroit, died on Aug. 10, 1954, at the age of 59. Dr. Beaver was a founding fellow of the College of American Pathologists. He was a member of the American Association of Pathologists and Bacteriologists and the American Society of Clinical Pathologists. He was a founder, and at the time of his death was president, of the Detroit Institute of Cancer Research. For many years he had been a member of the administrative board, and consultant of the Michigan Tumor Registry.

NOTICE

The Annual Meeting of the American Medical Association will be held in Atlantic City, June 6-10, 1955. Physicians hoping to take part in that meeting should communicate with their Section officers as early as possible. Deadlines have been set, after which applications will be considered only if there are withdrawals in the program.

DEADLINE FOR SECTION PAPERS, Dec. 15, 1954

DEADLINE FOR SCIENTIFIC EXHIBIT, Jan. 10, 1955

Communications should be addressed to the following:

Section on Pathology and Physiology

Secretary—Edwin F. Hirsch, M.D., 1439 So. Michigan Ave., Chicago 5, Ill.

Representative to Scientific Exhibit—Frank B. Queen, M.D., 3181 S.W. Sam Jackson Park Road, Portland 1, Ore.

SCIENTIFIC EXHIBITS

The A. M. A. Archives of Pathology is pleased to introduce in this issue a new feature called "Scientific Exhibits."

This feature attempts to preserve in more permanent form some of the instructive Scientific Exhibits that are presented at the Annual Meetings of the American Medical Association. Although thousands of physicians attend the Annual Meetings, viewing and studying the scientific

exhibits at first hand, other thousands are unable to attend. For the first time, the latter group is now given the opportunity to see the exhibits reproduced in the pages of the various A. M. A. Specialty Journals.

While it is at present impossible to publish all the Scientific Exhibits presented at each Annual Meeting of the American Medical Association, it is feasible to select a representative few for the purpose of introducing the new feature to the readers of this periodical. Should "Scientific Exhibits" be well received, it is planned to expand the new department in future issues of the A. M. A. ARCHIVES OF PATHOLOGY.

CORRECTION

The 10th line of the article, "Carcinoma of the Testes Metastatic From Carcinoma of the Prostate," by Drs. Saul Kay, G. R. Hennigar, and J. W. Hooper Jr., on page 121 of the February, 1954, issue of the ARCHIVES should read as follows: "Willis lists three pathways by which testes may receive metastatic tumors:"

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1. Kroop, I. G. and Shackman, N. H.: Proc. Soc. Exper. Biol. & Med. 86:95 (May) 1954.
2. Wood, H. F., and McCarty, M.: J. Clin. Investigation 30:616 (June) 1951.
3. Stollerman, G. H., et al.: Am. J. Med. 15:643 (Nov.) 1953.
4. Hedlund, P.: Acta med. Scandinav., Supplement 196:579, 1947.

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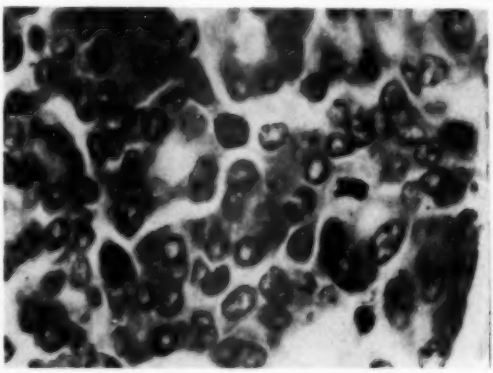

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